Meetings of the Maize and Sorghum Section of Eucarpia were held in:

1960  Rome
1962  Rome
1964  Vienna
1967  Montpellier
1969  Budapest
1971  Freising-Weihenstephan
1973  Zagreb
1975  Paris-Versailles
1977  Krasnodar
1979  Varna
1981  Montreux
1983  Warsaw
1985  Wageningen

For information on proceedings of these meetings, write to the Secretary, Eucarpia, P.O. Box 128, 6700 AC Wageningen, the Netherlands.
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I. Bos: Relative efficiency of direct and indirect mass selection for biomass yield
F.M. Engels & J.W. Cone: Structure and digestibility of stem cell walls of maize (Zea mays) grown at different temperatures
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Since 1960, scientific meetings of the Maize and Sorghum Section of Eucarpia, the European Association for Research on Plant Breeding, are held alternately in Eastern and Western Europe usually at 2-year intervals. This series of meetings of breeders and scientists is a continuation of a series of meetings on hybrid maize organized by FAO in the 1950s.

During the 12th Meeting of the Section, in Warsaw in 1983, the Foundation for Agricultural Plant Breeding (SVP) at Wageningen was invited to organize the next meeting. This proposal was accepted. The Organizing Committee, including representatives of research institutes and breeding companies, started work in autumn 1983.

It was decided to focus the scientific part of the Congress on one main theme ‘Breeding of Silage Maize’ and to invite speakers. All other scientific contributions, including subjects not related to the main theme could be presented in posters. We had in mind a thorough treatment of specific aspects of breeding of silage maize for an area too cool for grain production.

In all, 139 scientists and breeders from 25 countries participated in the meeting, which started on Monday September 9 with an opening address by Dr Z. Królikowski, President of the Section, and an address of welcome by Ir M. Kamps, President of the Dutch Plant Breeders Association (NKB). After an introductory paper on ‘Maize in the Netherlands’ followed four days of sessions on ‘Use of genetic resources’, ‘Cold-tolerance’, ‘Improvement of feeding value’ and ‘Selection criteria for silage maize’. Each session closed with a panel discussion. The programme was interspersed with poster presentations, a 1-day excursion to Dutch breeding companies (Cebeco-Handelsraad, Van der Have BV, Zea Select BV and Zelder BV), and a ½-day excursion to institutes in or near Wageningen (Foundation for Agricultural Plant Breeding, SVP; Governmental Variety Testing Institute, RIVRO; Institute for Application of Atomic Energy in Agriculture; ITAL, and Institute for Horticultural Plant Breeding, IVT). The programme included a reception by the Ministry of Agriculture and Fisheries, and a congress dinner at Castle Doornenburg. The Congress was closed with sessions reporting on and discussing the activities of the working groups of the Section and a session on Section organisation. The main conclusions of this last session were as follows. The next Congress will be organized by the Maize Research Institute in Trnava, Czechoslovakia. The work of the Northern and Southern Committee will be continued. An attempt will be made to revitalize the Sorghum Committee. Dr L. Kojić (Yugoslavia) was elected as the new chairman of the Southern Committee, and Dr O. Dolstra (Netherlands) and Dr M. Neštický (Czechoslovakia) were elected for the next period as President and Vice-President of the Maize and Sorghum Section of Eucarpia.
On behalf of the Organizing Committee, we would like to thank everybody concerned in the organization, especially the hosts of the excursions, and several colleagues at the Foundation for Agricultural Plant Breeding (SVP) and at the International Agricultural Centre (IAC).

We also gratefully acknowledge financial support by the following organizations:
- Ministry of Agriculture and Fisheries, The Hague
- Dutch Breeders Association (NKB), Amersfoort, representing the following breeding companies
  Cebeco-Handelsraad, Vlijmen
  SES Holland BV, St Annaparochie
  D.J. van der Have BV, Rilland
  Zea Select BV, Groningen
  Zelder BV, Ottersum
- European Association on Plant Breeding Research (Eucarpia), Wageningen
- Netherlands Convention Bureau, Amsterdam
- Agricultural University, Wageningen
- Commodity Board for Agricultural Seeds, The Hague

Finally, we would like to thank editorial staff of Pudoc in particular Mr. J.C. Rigg, for its active role in the realization of these Proceedings.

The editors
Opening session
Maize for silage in the Netherlands

H.A. te Velde

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Summary

Maize growing for silage has extended considerably in the Netherlands. This has required attention to details of cropping, in yield over the years, digestibility, losses during ensiling, factors in yield stability, seed quality, severe cold testing, varietal testing, components of variance, coefficient of variation and the importance of including in the Descriptive List of Varieties for the choice of a silage maize variety.

Descriptors: silage maize, Zea mays L., Netherlands, dry matter content, yield, yield stability, digestibility, seed quality, cold testing, varietal testing, trial error

Area

The Netherlands has a total land area of 3.7 million hectares (37 000 km²). Two million hectares are farmland, of which 59% for grassland, 35% for arable farming and 6% for horticulture. In 1984, the area under maize for silage (166 200 hectares : 1662 km²) accounted for 23% of the area under arable crops and so for the first time maize became the main cultivated field crop in area. Of all the silage maize, 80% was grown on sandy soils.

The production of maize for grain and maize for corn cob mix is very restricted. In 1984, about 400 ha were sown for this purpose. The area under silage maize increased slowly in the 1960s but rapidly since 1970 (Fig. 1).

Reasons for the considerable extension in silage maize production

The most important reasons for the considerable extension are as follows.

1. Conversion of the mixed farm (arable farming, grassland management, and stockfarming). Mixed farms were often small and mostly situated on sandy soils. For reasons of economy, they have been advised to dissociate and specialize in one type of farming, or to give up farming. Many farmers specialized in dairy farming and fodder production, and some in beef production. In 1983, just over half the area under silage maize was grown on these farms. About a quarter was grown for sale on pig farms, poultry farms and veal units, which purchase all the feed they require for their own stock; 22% was grown on arable farms (CBS, 1983). The silage maize from these four sectors of farming is sold to livestock farmers. So silage maize is not only a fodder crop but also a commercial one.

2. The farms with livestock have a high slurry output. Maize grows well with a large amount of slurry (Dilz et al., 1984); often, however, it is used to excess, which imposes a burden on the environment.
3. Maize is a highly self-tolerant crop and there is a lot of monoculture. Half of the area under silage maize is situated in regions where it takes up 84% of the area under arable crops. On average, 1 ha of these sandy grounds is used for the production of silage maize, against 4 ha for grassland. The rotation between arable crops and grassland is very limited.

4. Since the 1960s, labour productivity in drilling of maize, weed control, harvesting and ensilage have increased considerably thanks to improved machinery. In practice, this has been exploited by letting out almost all operations to contractors, who usually came from the farming community. In 1984, the average area under silage maize per farm amounted to 3.8 hectares; on two-thirds of the farms, this area was smaller. The average drilling or harvesting area per contractor amounted to 197 ha. In general, growing of silage maize demands little labour. Some stockfarmers make arrangements with the contractor for growing and harvesting by phone, the same way as they order feedstuffs. Usually the farm is sufficiently mechanized for the provision of silage maize to the cattle.

5. Maize breeders concentrated more on the breeding of silage maize varieties.

6. By the 1960s, it was known that high dry matter yield could be obtained with efficient growing methods and reasonably good weather. Becker (1976) optimized cropping practices on fields that are highly suitable for maize. Moreover, he based yield on a varietal purity of 100%; crop sampling for chemical analysis was therefore selective, i.e. without inbred plants or possibly inbred plants. So yield might have been a little high in comparison with real yields which included some inbred plants. From 1965 to 1969, the four best varieties yielded 16.0 tonnes of dry matter per hectare per year. In the same period, the same varieties yielded 12.9 t/ha in the official variety testing trials on other fields with methods that were then common practice. From 1970 to 1971, average dry matter yields of the four best varieties in the two types of trials amounted to 17.3 and 14.7 t/ha, respectively. After these results and experience
gained in maize study groups the Agricultural Advisory Service could recommend an extension of the total area under silage maize.

7. The Netherlands was not the only country to extend production of silage maize considerably. This trend was international. Originally silage maize was more commonly produced further inland in France and West Germany, but production gradually extended westwards to Brittany and Lower Normandy and northwards to Belgium, the Netherlands, and northwestern Germany (Table 1). In Denmark, silage maize was introduced recently; in England and Wales, however, production has been halved since 1977. In westernmost France, silage maize represented about 35% of the area under arable crops in 1984. For the Netherlands as a whole, this was almost a quarter, but in the southern and eastern sandy areas the share was a little more than 70%. The considerable extension of silage maize boosted its share in the national roughage ration for indoor feeding from 15% in the winter of 1975/1976 to 28% in 1983/1984.

Cropping practice

Silage maize is sown exclusively in tilled soil, preferably between 20 April and 5 May; earlier sowing hardly ever occurs, but later sowing does. Many fields have a compact subsoil. This obstructs drainage and results in later sowing dates and in lack of moisture in summer. In general the aim is, 95 000 to 100 000 plants per hectare. Usually the farmers expect 5 to 10% of the seed not to emerge, though often many more seeds do not produce satisfactory plants. This can be due to a poor seedbed, to drilling too deep and occasionally too superficially, and to poor seed.

The average growing season extends about from 1 May to 10 October. The time-temperature sum above 6 °C (Vis, 1981) amounted for the southern and middle part of the Netherlands to about 1400 °C d and for the northern part to about 1280 °C d. This corresponds to about 2720 and 2520 Ontario heat units, respectively.

Average precipitation in May-September is 340 mm, varying from 160 to 460 mm. Lack of

<table>
<thead>
<tr>
<th>Country, region</th>
<th>Relative area (%)</th>
<th>Area (km²)</th>
<th>Share of area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>France</td>
<td>22</td>
<td>64</td>
<td>14 010</td>
</tr>
<tr>
<td>Pays de Loire</td>
<td>16</td>
<td>53</td>
<td>3 370</td>
</tr>
<tr>
<td>Basse-Normandie</td>
<td>9</td>
<td>67</td>
<td>1 400</td>
</tr>
<tr>
<td>Bretagne</td>
<td>5</td>
<td>58</td>
<td>3 100</td>
</tr>
<tr>
<td>West Germany</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>south part</td>
<td>21</td>
<td>61</td>
<td>5 094</td>
</tr>
<tr>
<td>north part</td>
<td>4</td>
<td>33</td>
<td>3 599</td>
</tr>
<tr>
<td>Belgium</td>
<td>7</td>
<td>50</td>
<td>1 080</td>
</tr>
<tr>
<td>Netherlands</td>
<td>3</td>
<td>47</td>
<td>1 660</td>
</tr>
<tr>
<td>east + south; sand</td>
<td>5</td>
<td>51</td>
<td>1 160</td>
</tr>
<tr>
<td>Denmark</td>
<td>0</td>
<td>3</td>
<td>210</td>
</tr>
<tr>
<td>England &amp; Wales</td>
<td>6</td>
<td>160</td>
<td>160</td>
</tr>
</tbody>
</table>
rain should be compensated by supplementary soil moisture. Irrigation is only seldom used.

In the Netherlands, silage maize is widely grown in monoculture. Pratylenchus and Tylenchorrhynchus nematodes can then spread and do damage. The use of pesticides does not seem profitable. More than 100 tonnes of slurry per hectare reduces the number of nematodes considerably. Moreover, in monoculture fungi can cause browning of the roots. This can be prevented by injecting fungicides into the soil, but this solution is not used in practice. Weed control can be complicated by prevalent pernicious weeds. If, however, monoculture is practised properly, silage maize yields do not necessarily decrease (Maenhout, 1984). For weed control, triazine herbicides and some others are essential. Some pernicious weeds, however, cannot be controlled by relatively cheap triazine herbicides. Other weeds have become resistant to these herbicides. Increased mechanical weed control or a mixture of different herbicides seem effective.

Insects hardly do damage, though fungi do: stalk rot (Fusarium spp.) and smut (Ustilago maydis).

**Characteristics determining value of silage maize varieties**

It is expected that ever better-producing maize varieties will be developed, which will not lodge at harvest. The dry matter content should be sufficiently high to reduce silage losses. Moreover, the varieties should be stable in yield and not incur extra costs. These are the starting points for official varietal testing, for which varieties are grown according to commercial practice by good farmers. The only differences are the slightly greater rate of sowing and the thinning of the plants to an equal density of each variety, mostly 10 plants per metre.

**Dry matter yield** Yields from silage maize varietal trials are known from 1954 onward. The average dry matter yield of the recommended varieties in 1965-1984 are given in Figure 2. Yields before that period have been given by te Velde (1984b). Figure 2 also gives part of the calculated trend in yields since 1954. The highest correlation coefficient was developed with a
third-degree regression curve: \( r = 0.67 \). The average annual yield increase amounts to about 150 kg of dry matter per hectare, of which 74 is due to better varieties.

In 1981 and 1982, the best new variety, now recommended, yielded 21.3 and 22.2 tonnes of dry matter per hectare, respectively, on the field with the highest yields. However in the same years, varietal trials in subtropical Italy resulted in record yields of 32.0 and 33.7 t/ha, respectively. (Italy, 1982; 1983).

Record yields on Dutch farms amounted to 20 tonnes of dry matter per hectare. The average yield in 1980-1984 was 13 t/ha.

**Yield of nutrients**  Almost all the forage maize is supplied to cattle as silage. It is an energy-rich feedstuff and contains structural carbohydrates, which are essential for good rumen fermentation of cattle. The content of structural components in silage maize is about half of that of hay and grass silage, because grain scarcely contains any.

Silage maize also contains some protein, which partly meets the protein need of cattle and partly provides energy. There is little difference in protein content between varieties. Protein content has no bearing on the agricultural value of a variety.

Emphasis is placed on yield of net energy for cattle, which depends on dry matter yield, organic matter content, and content in digestible organic matter, loss during ensiling and feed refused by the livestock. Between varieties there was no difference due to feed intake, which is therefore ignored.

**Energy in the organic matter**  In the Netherlands, energy content in dry matter or in digestible organic matter is usually defined in terms of net energy for milk production in the arbitrary unit VEM, equal to 6908 J. The VEM is close in value to net energy for milk production of 1 g of barley (van Es, 1978).

The net energy content of the varieties in official trials is estimated in vitro with rumen liquid and reference materials whose digestibility by sheep is known. According to Andrieu (1984) the digestibility in vitro of the organic matter of silage maize containing between 23 and 37% of dry matter is independent of the dry matter content.

The net energy content for cattle of silage maize depends in the Dutch climate on the mass fraction of hard grains or particles excreted undigested. So net energy for cattle may be less than for sheep. Data collected by Meijer & Steg (1984) suggest that after customary harvesting methods digestibility decreases for cattle by about 0.5 percentage unit for each percentage unit of dry matter content in the range of dry matter content 28-35%. The dry matter content of the grain will probably be a better criterion than that of the silage.

Obvious differences exist between the varieties in digestibility of organic matter and thus in net energy content for milk production (Deinum & Bakker, 1981). On the whole, relative differences have been almost constant over years. The relative difference between the recommended varieties of 1985 in net energy content in dry matter is 5% with LG 11 topping the list and Markant at the bottom. Reduced digestibility can be due to reduced digestibility of one or more parental inbreds.

**Dry matter content and loss during ensilage**  Loss during ensilage is a result of microbial activity and, if the fodder is too moist, also of effluent. Loss as effluent from the heterogeneous product silage maize was largely associated with the dry matter content of the whole crop: in trials, the negative correlation was strong \( r \leq -0.86 \). The dry matter content of the stover (leaf and stalk) appeared to be less relevant. In research and extension on green fodder crops
for silage, the relative loss is generally dependent on the dry matter content of the whole crop if no additive is applied. There seems to be no reason to assume that silage maize behaves differently.

In favourable weather for silage maize, the bulk can be harvested when the dry matter content is sufficiently high not to incur heavy loss during ensilage. The only difference between the varieties is then the difference in harvesting dates. In autumn, the content of dry matter usually increases by 1 percentage unit in about three days.

In the northern part of the Netherlands and in cold and often also moist summers, which have occurred three times during the past 15 years, the situation changes. The maize should then be ensiled at a lower than desirable dry matter content. Loss during ensilage is then much higher for later varieties than for earlier ones. The lower the dry matter content at ensilage, the greater the loss. The greatest difference in dry matter content of the recommended varieties of the 1985 List of Described Varieties amounts to 4.2% when all are harvested at the same time. If an early and relative late variety are ensiled at a dry matter content of, for instance 23 and 19%, respectively, because of unfavourable weather, difference in loss during ensilage of the latter is about 6% in favour of the early variety. At a dry matter content of 32 and 28%, respectively, the difference in loss is only slightly more than 2%. Because of potential differences in loss during ensilage, silage maize varieties in the Descriptive List of Recommended Varieties of Field Crops have been divided into three groups according to dry matter content. In association with region, earliness of the crop, sowing time and harvesting time, the greatest demand is for the varieties of the earlier two groups.

Dry matter content of the whole crop and grain maturity

Harvesting at a dry matter content of 28 to 30% of the whole maize crop seems best in order to make clamp silos to a depth of 1-2 m (ten Hag et al., 1984). Unfortunately, the dry matter of silage maize is difficult to estimate correctly just by observing the crop. A preferable criterion is grain maturity. However differences in dry matter content of stover can be large between varieties. Table 2 gives an example for three varieties. Reckoned by grain and ear, Keo is markedly earlier than LG 11, but the dry matter content of stover and of whole crop is markedly lower. In the Netherlands, little experience has been obtained with 'Keo', in contrast to 'Goal', which has a moister stalk. For grain maturity, 'Goal' was overrated relative to LG 11: effluent sometimes exuded even from the trailer during transport. Should the moisture content of the stover be taken into account in timing of the harvest to limit loss during ensilage? There may then be a higher loss due to undigested hard grains or particles in the faeces of cattle. A relative low dry matter content of the stalks and leaves is an undesirable characteristic.

Table 2. Maturing characteristics of three maize varieties. Dry matter contents for a characteristic without a letter in common are significantly different for a least significant difference of 0.05.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Keo</th>
<th>LG 11</th>
<th>Goal (=But 234)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAO index</td>
<td>180</td>
<td>260-270</td>
<td>240-270</td>
</tr>
<tr>
<td>DM content of ear (%)</td>
<td>54.5a</td>
<td>53.8ab</td>
<td>53.4b</td>
</tr>
<tr>
<td>DM content of stover (%)</td>
<td>20.3b</td>
<td>21.8a</td>
<td>20.5b</td>
</tr>
<tr>
<td>Share of stover in fresh matter (%)</td>
<td>73</td>
<td>69</td>
<td>73</td>
</tr>
<tr>
<td>DM content in whole crop (%)</td>
<td>29.9b</td>
<td>31.98</td>
<td>29.6b</td>
</tr>
</tbody>
</table>
**Sequence in maturity**  In practice and also in varietal trials, silage maize has been harvested in recent years at a dry matter content of 20-37%. This can influence the sequence in maturity. Table 3 gives an example. Shown is the relation 50% silking and dry matter content at harvest of four varieties in three years. In those years, the sequence in maturity of Clipper, LG 11 and Splenda is the same. In 1982 with favourable growing conditions and mid-harvest on 23 September, the variety Gracia was early, significantly earlier than Splenda. In 1983 with more normal weather conditions and mid-harvest on 21 September, Gracia was a rather early variety. In 1984 with bad weather and mid-harvest on 15 October, Gracia was a fairly late variety, significantly later than Splenda. Gracia is the most dent-like hybrid of the four.

The different responses of the varieties to growing conditions in 1979-1984 gave moderate average correlation between silking date and the dry matter content at harvest: \( r = 0.60 \). The sequence of maturity of some varieties is not stable to the average of the recommended varieties.

**Yield stability and avoidance of unnecessary costs**  The following varietal characteristics have unfavourable effects on yield stability and perhaps on production costs.

1. Too little resistance to lodging. More than 5% lodged plants at harvest is considered harmful because it results in capacity loss at harvesting, which incurs extra costs. Sometimes, the harvestable yield is reduced as well. In our windy climate, root or stem weakness are considered worse than susceptibility to stalk rot. Injurious consequences of the latter can often be prevented by timely harvesting, i.e. at a dry matter content of about 27%.

2. Slow initial growth. This can be the result of low-quality seed, but is often a varietal characteristic. For the twelve recommended varieties for 1985, there was a reasonable correlation between score for initial growth and the relative yield of dry matter yield of the varieties in 1979-1984 (\( r = 0.78 \)).

3. Susceptibility to cold in early summer, which often occurs in the second half of June. If so, varieties with moderate resistance lag on the average 3% in yield behind varieties which are very cold-resistant.

4. Susceptibility to rust (*Puccinia* spp.). If rust is widespread, yields can be disappointing. This has sometimes happened for new varieties during testing.

5. Susceptibility to smut (*Ustilago maydis*). Smut damage depends on the extent and site of infection. Severe attack of the ear is most harmful. In a 1982 varietal trial, one variety was 38% smut-infected, mainly in the ear. The dry matter yield was 9% below what might have been

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Table 3. Silking dates and dry matter content of the whole crop at harvest of four varieties in three years, with markedly different weather. Dry matter contents in a certain year without a letter in common are significantly different for least significant difference of 0.05.

<table>
<thead>
<tr>
<th>Year</th>
<th>Characteristic</th>
<th>Clipper</th>
<th>LG 11</th>
<th>Splenda</th>
<th>Gracia</th>
</tr>
</thead>
<tbody>
<tr>
<td>1982</td>
<td>50% silking</td>
<td>17 July</td>
<td>19 July</td>
<td>22 July</td>
<td>24 July</td>
</tr>
<tr>
<td></td>
<td>DM content (%)</td>
<td>35.6a</td>
<td>34.0b</td>
<td>33.2a</td>
<td>35.7a</td>
</tr>
<tr>
<td>1983</td>
<td>50% silking</td>
<td>28 July</td>
<td>30 July</td>
<td>2 Aug.</td>
<td>4 Aug.</td>
</tr>
<tr>
<td></td>
<td>DM content (%)</td>
<td>32.5a</td>
<td>30.9b</td>
<td>28.5c</td>
<td>29.8c</td>
</tr>
<tr>
<td></td>
<td>DM content (%)</td>
<td>27.4a</td>
<td>25.4b</td>
<td>24.1c</td>
<td>22.9c</td>
</tr>
</tbody>
</table>

1. 1, flint; 5, dent.
expected and the dry matter content was 3% below. In 1979-1983, on the average there was an
absolute reduction of 1.5% dry matter yield and 0.2% in dry matter content for every 10%
smut-infected plants, including small galls. Cook (1978) mentions a slightly higher loss of dry
matter yield. Burgstaller et al. (1977) observed that silage made from smut-infected maize
caused no injury to cattle. Varieties clearly differ in susceptibility to smut. In practice, smut
damage can only be restricted by sowing varieties highly resistant to smut.

6. All varieties are susceptible to stress by drought. Drought reduces production of dry
matter; there are only small differences between the recommended varieties. The grain set can
be poor, although it obviously differed between varieties. This difference was not related to
the normal order of silking dates. In 1982, there was much drought damage in a trial field. The
average yield of dry matter amounted to 11.8 t/ha, at least 25% less than other trial fields,
which had been much less affected by drought. Groups of varieties had a grain set close to 48,
26 and 5%, respectively. At a grain set of 5%, the dry matter yield was 2% lower than at a
grain set of 26 and 48%; also the net energy content in dry mater was only 2% lower. Pre­
viously Deinum & Knoppers (1979) observed a 4% difference in net energy content with a
good seed set and no seed set, respectively, of crops with a very high and a high dry matter
yield, respectively. De Jong (1984) mentions that the nutritive value of early and extremely
wilted maize without grains was at most 10% lower. Consequently, poor seed set or no set due
to drought is not as disastrous for silage maize as it is for grain maize.

7. Susceptibility to environmental effects in comparison to winter rye. Winter rye used to be
the most grown crop in the southern and eastern sandy regions of the Netherlands. Silage
maize is now most important. During the past fifteen years the average yields for rye and for
silage maize have been, according to crop estimates, at most 14% above the linear trend; for
rye, 14% lower yields have been recorded and for silage maize, 22%. The average annual
yields of silage maize varietal trials too have shown these fluctuations with 22% lower yields in
1976 and 1984, respectively (Figure 2). For silage maize, 1976 was hot but much too dry; 1984
was cold and in autumn too wet. Apparently silage maize is more susceptible to less favourable
growing conditions than winter rye.

8. Susceptibility of varieties to environmental effects. Yield of a recommended variety can
be better or worse in a given year than anticipated in relation to the yield of the other recom­
manded varieties. Of all varieties recommended for more than five years, ‘Capella’ and ‘Dor­
ina’ have been most stable with maximum fluctuations of 2%, followed by LG 11 with 3%
extreme differences. As yet, Fronica has fluctuated between –4 and +3% and Brutus between
–3 and +5%. Better or worse yields are often an effect of seed quality.

High-quality seed is indispensable

During the most favourable sowing period for maize, the average soil temperature at the
depth of drilling is often 8-9 °C. Usually this temperature rises slowly but sometimes it remains
at this level for a few weeks. So high demands are made on seed quality. A germination
capacity of at least 90% by the ISTA (International Seed Testing Association) method does
not give enough information. A severe cold test gives much better information (Ebskamp,
1983). However no cold test has yet been developed that is reproducible world-wide. Even
with good control of temperature and moisture content of the soil, the result of the test also
depends on the type of soil and the pathogens present. Furthermore, there is the uncertainty in
interpretation through changing field conditions and their influence on the field emergence of
maize. A correct estimate of field emergence is essential because in general the optimum
number of plants of silage maize is 95,000-100,000 per hectare (9.5-10 per square metre). Figure 3 illustrates results of a severe cold test by RIVRO and the emergence of silage maize on fields in the southern part of the Netherlands. With early drilling, field emergence is influenced by field conditions. Site W has a relatively warm soil and C a rather cold soil with good moisture retention. Of the three sites, the dry matter yield of the standard varieties was highest on C, i.e. 19 t/ha against about 16 t/ha at the other two sites, when sowing took place in April. Site N has a normal soil, for soil temperature. Seed was sown there on two dates. Later drilling resulted in better emergence; this is often so, for instance in 1979 (te Velde, 1984a). Moreover Figure 3 shows that it is much easier to estimate field emergence of maize from top-quality seed than from seed which, according to the cold test, has less vigour.

In the applied cold test, the RIVRO cold test, seed treated with a fungicide is sown in sandy soil with about 3% organic matter, previously cropped with maize and with moisture content 60% of the field capacity. The seed is maintained at a temperature of 8.5 °C for 18 days. After those eighteen cold days, the temperature is increased to 27 °C for three days (Ebskamp, 1983). For the varieties conditionally accepted to the official variety trials, RIVRO has the past few years interpreted the results of this cold test as follows:

<table>
<thead>
<tr>
<th>Emergence in the cold test</th>
<th>Seed quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>more than 90%</td>
<td>good to very good</td>
</tr>
<tr>
<td>80 to 90%</td>
<td>reasonably good</td>
</tr>
<tr>
<td>70 to 79%</td>
<td>doubtful</td>
</tr>
<tr>
<td>less than 70%</td>
<td>unacceptable</td>
</tr>
</tbody>
</table>

The amount of seed required can be predicted more accurately if the seed quality is good and the genetic potential of the variety is shown to better advantage. Varieties that scored lower in the cold test produce weaker plants and lower yields. For instance in a trial with an
equal plant density obtained by using a little too much seed and thinning to 10 plants per square metre, maize from a batch with a 92% field emergence yielded 8.5% more than maize of the same variety from a batch with 80% field emergence \((P < 0.001)\). In practice, it is exceptional that more seed of weaker batches is used. Seed quality as indicated by a cold test is an essential yield-determining factor (Boer, 1984).

The following are major causes of disappointing emergence in a cold test in comparison with the germination capacity by the ISTA method: damage to the pericarp, old seed, frost damage and genetic characteristics. Damage to the pericarp can be caused by artificial drying too fast or by handling of the seed too roughly for that particular variety (van der Putte, 1985; Navratil & Burris, 1984). Processing is of course different for each variety. Quality control should obviously play a major role in seed processing from the moment of harvesting. Further, seed treatment must not have injurious consequences as happened with lindane (van der Werf et al., 1985).

**Official varietal testing**

In the official varietal testing the following points are of interest:

1. The official VCU testing (Value in Cultivation and Use) of varieties of maize for silage is carried out by RIVRO on behalf of the Committee for the Compilation of the List of Varieties of Field Crops. The assessment by this Government Committee of three persons is independent.

2. The Committee makes its judgement after three or sometimes four years of trials. A new variety, whether bred by a foreign or by a Dutch breeder, has then to be entered into the Netherlands Register of Varieties, so the new variety must be clearly distinguishable from the other varieties, must be sufficiently homogeneous and must be stable in its characteristics. The examination for this is also by RIVRO. To enter the List, a new variety has to be an improvement in the total range of the described varieties. All the described varieties are used as standards in the test. The varieties are classified in a few maturity groups.

3. The General Netherlands Inspection Service for Seed of Field Crops and for Seed Potatoes (NAK) supplies the necessary seed (20 kg per variety) of the standards to RIVRO after sampling several commercial batches.

4. The new varieties must already have been pretested in the Netherlands by the breeders. Since 1985, they do this in standard trials with four standard varieties indicated by RIVRO. The trial results must be submitted to RIVRO.

5. Not all the new varieties remain under test for three years, only the varieties that still have a chance. About two-thirds of the number of varieties are already dropped after the first year of testing.

6. Of all hybrids tested for the first time in 1972-1981, 9% has been accepted into the Descriptive List.

7. To come to decisions, it is necessary to compare the varieties as well as possible. To be able to register even small differences with a reasonable reliability, a small relative error is necessary. Therefore, trials are laid out on fields reasonably uniform in fertility. On sandy soils, where most trials are situated, such fields are rare.

**Trial error** For trials over several years, the error consists of three components: the error for the trial, the interaction variety \(\times\) site and the interaction variety \(\times\) year. Figure 4 shows the components of variance \((\sigma^2)\) over five (4 in 2 years) trials per year with four replicates per trial.
field during 1, 2, ..., 8 years. The statistical treatment applied for the yields is an iterative version of 'fitting of constant methods'. This is a standard method in varietal research in the Netherlands. After such a treatment, the varieties can be compared among one another, even though they have been tested in different years.

In Figure 4, the variances during the trials are somewhat smaller than the interaction variety × site as the number of years of trial increases. They regularly decrease as they refer to more years of trial. The variance due to the interaction variety × year is markedly higher than for the other two components separately, but decreases up to five years of trial. After five years, there is again an increase. This is in particular because in the sixth trial year (i.e. 1982) the yield of a variety recommended for the first time was very disappointing in comparison with the three previous trial years. In the following year, the yields of all four varieties which were recommended for the first time were disappointing, one of these was very disappointing indeed. This resulted in an even greater variety × year interaction. For the cropping year 1984 – the eight year in Figure 4 – no new silage maize varieties were included in the List of Recommended Varieties and the varieties included the previous year better met expectations. After eight years, the interaction variety × years could therefore be less than after seven years.

That is the current situation. If, however, only the six varieties were taken into account that have been included in the List of Varieties from the first two years onward it becomes apparent that $\sigma^2$ for the interaction variety × year decreases just as regularly as $\sigma^2$ for both other components of the total variance, only at a slightly higher level. After three years, this $\sigma^2$ was 1.15, and after eight years 0.7.

To gain an insight into total variance, the three separate variances should be added. Table 4 does this and gives the corresponding coefficients of variation as well.

Coefficients of variation for the yields of trials for more than one year have been low. They were lowest after four and five years. Fairly small differences in yield are detectable fairly soon with reasonable reliability. The greatest inaccuracy when estimating the relative yield of a variety for the following year does not result from too few replicates per trial field or from too few trial fields per year, but from a large interaction variety × year. Three years seems a necessary minimum. The interaction variety × year can be smaller, especially by supplying better seed in the first year of recommendation than was done in 1982 and 1983.
Table 4. Total variance of dry matter yield (kg m$^2$ are$^{-1}$) ($\sigma^2$) and coefficients of variation (CV) corresponding to annual dry matter yield of five silage maize varietal trials (four fields in 1979 and 1983) in four replicates per field after one, two, three, ... eight years of trial.

<table>
<thead>
<tr>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>$\sigma^2$</td>
<td>5.69</td>
<td>6.82</td>
<td>3.83</td>
<td>2.87</td>
<td>1.83</td>
<td>2.17</td>
<td>2.35</td>
<td>1.73</td>
</tr>
<tr>
<td>CV</td>
<td>1.64</td>
<td>1.71</td>
<td>1.26</td>
<td>1.08</td>
<td>0.84</td>
<td>0.90</td>
<td>0.94</td>
<td>0.88</td>
</tr>
</tbody>
</table>

The Descriptive List of Varieties in choice of variety

There is free commerce for all varieties included in the Common List of Varieties of Arable Crops of the European Community within the Netherlands. The List includes a considerable number of early varieties. About 97% of the cultivated area under silage maize, however, is sown with a variety from the Descriptive List of Varieties. For 1985, this List included 12 varieties, 5 of which occupied 78% of the area (CSRL, 1984). Varieties have been included for an average of 8.8 years. Some varieties have been included only for three years because the breeder supplied seed less suitable for Dutch conditions. To operate on the Dutch seed market, it is thus essential to have one or more varieties included in the Descriptive List of Varieties of Field Crops and to supply seed with good vigour in a cold seedbed.

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CBS (Centraal Bureau voor de Statistiek), 1983. Maandstatistiek van de Landbouw 31, 10: 34.


Use of genetic resources
Use of exotic sources of germplasm for maize improvement

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Summary

Use of exotic germplasm in maize (Zea mays L.) breeding programs in temperate zones is extremely limited. Although there is a definite need to broaden genetic diversity, breeders are probably sampling only about 5% of the available races of maize. Much of the exotic maize germplasm, particularly that of tropical or semitropical origin, is not well adapted and performs poorly in temperate zones. However a few research programs are directing significant resources into the evaluation of exotic germplasm for breeding potentials (including combining abilities and heterotic responses with adapted materials) in temperate regions. Data from these studies have demonstrated that subjective evaluations based on characteristics such as ear and kernel types of races per se may show little relationship with the inherent combining ability in hybrids or test crosses. With these data too, several collections have been identified as promising sources of germplasm and a number of collections have been used for the development of some widely divergent breeding populations. Many of these populations show superior genetic potential for yielding ability. However traits such as plant height, ear quality, and lodging resistance will require improvement before most of the populations are acceptable in commercial breeding programs for grain yield. Some may be suitable for silage purposes, however.

Descriptors: maize, Zea mays L., races of maize, exotic germplasm, maize improvement, genetic diversity, maize germplasm stocks, exotic test crosses, selection procedures

Introduction

In most plant breeding programs, improvement is accompanied by a decrease in genetic diversity, particularly in the materials that reach commercial production. This reduces the potential variability available to the breeder for selection and, more importantly, the commercial product has increased vulnerability and faces an increased risk of economic loss from new pathogens, insect pests or unusual environmental stresses. Those of us from the United States recall vividly the anxiety created in the maize (Zea mays L.) industry when an epidemic of a previously minor disease, southern corn leaf blight, struck in 1970. The lack of diversity in that situation was associated with the cytoplasm. Recent surveys of the use and variation of publicly developed inbred lines from the United States and Canada indicate that nuclear diversity in commercial maize is limited when compared with the total variability that exists in the species (Zuber & Darrah, 1980; Stuber & Goodman, 1983a). I would expect that surveys of European maize would show similar patterns of variation. Thus it seems prudent that efforts should be made to widen the germplasm base in maize breeding programs.

Although the term exotic may have several meanings, exotic maize germplasm in the Unit-
ed States and Canada is usually considered to include all sources of unadapted germplasm: domestic unadapted, temperate foreign, and tropical or semitropical. In this broad interpretation, there has been considerable use of exotic germplasm in experimental research programs and in the farmers' fields. However a survey by Goodman (1985) shows that only about 4.4% of the United States maize area (which totals about 32 400 000 ha) is being planted with hybrids containing any germplasm from outside the country (Table 1), and those hybrids generally consist of only 10-25% exotic germplasm. Thus, less than 1% of the germplasm used in commercial production in the United States originates from foreign exotic sources and only a fraction of that is from tropical exotic sources.

Brown & Goodman (1977) have indicated that the maize germplasm found in the New World can be classified into some 250 to 300 races, which are represented by about 25 000 accessions. As Goodman (1985) indicated, virtually all hybrid maize used commercially in the United States is derived from only one race, Corn Belt Dent. Commercial maize grown throughout the temperate zones of the world have been derived from races representing about 2% of the available germplasm. This germplasm includes the Corn Belt Dents, the Northern Flints, and the Cateto (Argentine) Flints. Even on a worldwide basis, most commercial maize is derived from only six major racial groups: the three listed above plus the Mexican Dents, Caribbean Flints, and the Tusons. This represents less than 5% of the available genetic resources. When all currently used (including unmarketed) breeding materials are considered, breeders in temperate zones are probably sampling about 5% of the available races of maize; on a worldwide basis, breeders are experimenting with probably no more than 10% of the races available (Goodman, 1985). Unfortunately the majority of even these materials will probably never reach farmers' fields.

Several reasons can be listed to account for the limited use in the United States and other temperate zones of the Northern Hemisphere, of exotic germplasm, particularly that of tropical or semitropical origin:

- Desirable characters are masked by adverse photoperiod responses. Thus, summer nurseries in temperate zones of the Northern Hemisphere are virtually useless for evaluating exotic performance per se and choosing materials for breeding use.
- Breeding efforts to improve landrace materials are fifty years behind those for currently used elite breeding materials.

Table 1. Summary of United States maize hybrids incorporating exotic germplasm in 1983. Adapted from Goodman (1985).

<table>
<thead>
<tr>
<th>Germplasm source</th>
<th>Number, of hybrids</th>
<th>Number of companies</th>
<th>Amount sold</th>
<th>Areas of adaptation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia</td>
<td>5</td>
<td>2</td>
<td>442 000</td>
<td>South; Corn Belt</td>
</tr>
<tr>
<td>Europe</td>
<td>14</td>
<td>5</td>
<td>290 000</td>
<td>North</td>
</tr>
<tr>
<td>South Africa</td>
<td>5</td>
<td>2</td>
<td>86 200</td>
<td>South; Corn Belt</td>
</tr>
<tr>
<td>Caribbean</td>
<td>8</td>
<td>2</td>
<td>94 100</td>
<td>Corn Belt</td>
</tr>
<tr>
<td>Cuba</td>
<td>4</td>
<td>1</td>
<td>45 500</td>
<td>South</td>
</tr>
<tr>
<td>Mexico</td>
<td>2</td>
<td>1</td>
<td>36 000</td>
<td>Corn Belt; North</td>
</tr>
<tr>
<td>Argentina</td>
<td>5</td>
<td>4</td>
<td>31 100</td>
<td>Corn Belt; North</td>
</tr>
</tbody>
</table>

1. One represents 80 000 kernels.
2. One hybrid (17 000 × 80 000 kernels) included both Cuban and South African parentage.
Linkages between favorable and unfavorable genes in crosses of exotic by adapted populations often exist and are perhaps not easily broken.

Little emphasis has been placed on the evaluation of the various sources of exotic germplasm. Thus there has been no good basis for choosing the best exotics for use in breeding.

When grown in the United States, most exotics exhibit one or more major weaknesses which include poor root systems; weak stalks; tall plants with high ears; tendency toward barrenness (particularly when crowded); susceptibility to maize smut; high moisture and slow dry-down of kernels. For those early exotics that will flower during summer growing seasons, yields of grain are low.

**Current status of major maize germplasm stocks**

The major maize germplasm banks holding accessions of most interest to plant breeders are shown in Table 2. Although some 25,000 accessions of maize are stored in these banks, effective utilization of these materials has been severely hindered for several reasons:

1. Detailed documentation of the several sets of collections is not available. Thus, determining what stocks are available, where they are and how to acquire them has been difficult for most maize breeders. Descriptions of the races are documented in Booklets on Maize Races (listed after the references), but they provide little information on traits of primary interest to the plant breeder and hardly any information on how to acquire the desired stocks.

2. Maintenance of the stocks has been extremely difficult at the germplasm banks because of inadequate funding. Much effort and resources are required to maintain and periodically multiply these stocks. Because funding has not been adequate, possibly 20% of the collections are no longer viable.

Although some accessions have already been lost because of inadequate maintenance, the United States Department of Agriculture has recently provided funds for a project to regenerate the maize collections stored in the major banks in Latin America. This project, under the direction of Dr. Major Goodman, will entail the growing of these collections in the areas where they are adapted. In addition to replenishment of seed lots stored in the Latin American germplasm banks, a sample of about 2 kg of each accession will be sent to the United States for storage at the North Central Plant Introduction Station (NCPIS), Ames, Iowa, and at the National Seed Storage Laboratory (NSSL), Fort Collins, Colorado. These accessions will then be available for distribution from NCPIS. It is anticipated that the following numbers of accessions will be regenerated each year until the project is completed: Mexico 1000, Colombia 800 and Peru 500.

Obviously, the effort demanding the highest priority now is the preservation of the maize germplasm that has not already been lost. Of equal importance, however, to this project is the planned publication of a catalogue listing the collections available, costs (if any) per sample, and a brief description of each collection including local name, race name, characteristics of collection site (e.g. latitude, longitude, altitude), source, kernel type and color.

The next step is to evaluate the various accessions (at least for a minimum number of agronomic descriptors) so that the maize researcher or breeder will be able to choose intelligently the particular germplasm source that might be appropriate for the needs of his research program. Fortunately, Pioneer Hi-Bred International (Johnston, Iowa) is providing funds over a five-year period for this project. Although the final plans for this project have not been completed, it is anticipated that evaluations will be made in the areas of Latin America where the
Table 2. Information on major germplasm banks for Latin American maize. From Goodman, 1983.

<table>
<thead>
<tr>
<th>Bank</th>
<th>Categories for information</th>
<th>telephone</th>
<th>areas covered</th>
<th>number of collections</th>
<th>number available for distribution</th>
<th>comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT)</td>
<td>Director, Maize Germplasm Bank CIMMYT Apdo. Postal 6-641 Londres 40 Mexico 6, D. F. Mexico</td>
<td>905-585-4355</td>
<td>Eastern South America, Mexico, Central America, West Indies</td>
<td>13 000</td>
<td>9 000</td>
<td>Bank of first choice when appropriate region is represented. Many composite and duplicate samples.</td>
</tr>
<tr>
<td>Instituto Nacional de Investigaciones Agricolas (INIA)</td>
<td>Maize Germplasm Bank Dr Francisco Cardenas INIA-Chapingo Apartado Postal No 10 Chapingo, Mexico</td>
<td>905-585-4555, ext. 5311 or 905-687-7421</td>
<td>Mexico, Guatemala, West Indies</td>
<td>9 000</td>
<td>6 000</td>
<td>Bank has better representation of individual Mexican and Guatemalan collections than CIMMYT.</td>
</tr>
<tr>
<td>Instituto Colombiano Agropecuaria (ICA)</td>
<td>Dr Fernando Arboleda ICA Apartado Aereo 51764 Medellin, Colombia</td>
<td>57-471-2459 or 57-471-1059</td>
<td>Venezuela, Colombia, Ecuador, Bolivia</td>
<td>6 000</td>
<td>5 000</td>
<td>This bank also theoretically houses Peruvian and Chilean collections, but neither are available for distribution.</td>
</tr>
<tr>
<td>Programa Cooperativo de Investigaciones en Maiz (PCIM)</td>
<td>Dr Ricardo Sevilla PCIM Universidad – La Molina Apartado 456 Lima, Peru</td>
<td>51-14-35-2035</td>
<td>Peru</td>
<td>4 000</td>
<td>2 900</td>
<td>The only source for Peruvian collections.</td>
</tr>
<tr>
<td>National Seed Storage Laboratory (NSSL)</td>
<td>USDA National Seed Storage Laboratory Colorado State University Fort Collins, Colorado 80521</td>
<td>303-484-0402/6418</td>
<td>Eastern South America. Colombia, Chile, Mexico</td>
<td>2800</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Some 2400 of these are foreign. Most represent early-maturing types, which can be reproduced in US. The remaining 600 collections represent the only comprehensive source for old US open-pollinated varieties, including US Indian corns.

These are standby collections, 2000 of which are Colombian and those are assigned Plant Introduction (PI) numbers. Many of the remainder are inaccessible. The Chilean collections are unique.
accessions are adapted, data for a minimum list of descriptors will be catalogued, and these
catalogues will then be available for all maize researchers and breeders.

Evaluations of exotic germplasm for genetic diversity and potential use in breeding programs

Although compilations of descriptions in the race booklets provide a subjective measure of
the diversity in available collections, a more objective appraisal of genetic variability can be
obtained with genetic markers such as isozyme (allozyme) alleles or chromosome knobs. McClintock et al. (1981) recently published the results of a comprehensive study of racial vari­
ation of chromosome knobs in maize, but a similar data set is lacking for comparison with
breeding materials.

Dr Major Goodman and I have been conducting a similar survey of genetic diversity and
racial relationships using allozyme loci as genetic markers. Nearly 1 000 typical collections
from Latin America have been surveyed. However only a small portion of the results have yet
been published (Goodman & Stuber, 1983; Stuber et al., 1977; Stuber & Goodman, 1983b;
1984). More than 406 inbred lines, mostly from the United States and Canada (Stuber &
Goodman, 1983a; Smith et al., 1984; 1985), and some commercial hybrids (Cardy & Kannen­
berg, 1982; Smith, 1984) have been surveyed by the same techniques. Therefore data are now
available for comparison of the variation among the various racial collections with that found
in currently used breeding germplasm. Because the allozyme technique is widely used com­
ercially too by several hybrid seed companies for cultivar identification and quality control,
further data on currently used inbred lines and other breeding materials will accumulate.

A measure of genetic diversity among sets of inbred lines and among typical collections of
Latin American races based on numbers of alleles at allozyme loci is presented in Table 3. In
these studies, variants at 23 allozyme loci were assessed, and thus for any set of germplasm at
least 23 alleles (one per locus) must be observed. The number or alleles in excess of 23 (Table
3) provides a measure of the diversity within and between different sets of material. For the
popular inbred lines, the variability ranges from about 5 to 15% (depending on the set of lines
chosen for comparison) of that found for the racial collections. Even for the set of 406 lines
(which includes sweet corn, popcorn, and Canadian flint lines, as well as white and yellow
dents), the number of alleles is only about 30% of that found in the racial collections.

An effort to evaluate a wide range of exotic collections of maize germplasm for potential
breeding values was begun about 10 years ago in my research program at Raleigh, North
Carolina (Stuber, 1978). Had it not been for the comprehensive set of typical racial collections
already assembled by Dr Major Goodman, this program would probably not been attempted.
However, Dr Goodman willingly provided his expertise and seeds from his collections for this
project. The program was expanded to include several testing sites in the southern United
States. Scientists at Tifton, Georgia; Mississippi State, Mississippi; College Station, Texas;
Columbia, Missouri; and Union City, Tennessee, cooperated in these investigations.

More than 600 collections were evaluated in this project. Tests were designed to identify
genetic potential for grain yield, for other agronomic traits, and for resistance to numerous
diseases and insects. Disease and insect pests included northern and southern leaf blights,
anthracnose, stalk rots, common and southern rusts, downy mildew, head smut, fall ar­
myworm, southwestern and European corn borers, and corn earworm. Because most of the
Latin American collections are poorly adapted to growing conditions in the United States,
largely because of adverse photoperiodic responses, evaluations of the races per se did not
seem practical, at least not in the initial evaluation phase of this project. Therefore, each racial
Table 3. Diversity observed among sets of inbred lines and among Latin American racial stocks of maize. The most popular lines (Sprague, 1971; Zuber, 1975; Zuber & Darrah, 1980) are those accounting for more than 5 000 000 kg of seed each, about 1% of United States annual needs. Eleven lines were represented in 1980, 12 in 1975, and 6 in 1980. The 39 popular lines are from Zuber and Darrah (1980). Each line accounts for at least 500 000 kg of seed each, about 0.1% of United States annual needs. Table adapted from Goodman & Stuber, 1980; and Stuber & Goodman, 1983a.

<table>
<thead>
<tr>
<th></th>
<th>Most popular lines</th>
<th>39 popular lines, 1980</th>
<th>406 Latin American races</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of alleles observed in excess of 23, for 23 isozyme loci</td>
<td>15</td>
<td>18</td>
<td>11</td>
</tr>
</tbody>
</table>

collection was test-crossed to three United States single-cross hybrids. These hybrids (only one had been used commercially) were chosen because they were known to be susceptible to numerous diseases and presumably would not mask desirable genetic factors in the races to be tested. Because of the photoperiod sensitivity of many of the exotic collections, many of the test crosses were made in the winter Florida nursery under short-day conditions. Most of the evaluations were made using crosses with only two of the tester hybrids.

A primary reason for crossing each collection on the single-cross testers was to bring maturity into a range that allowed a reasonably objective evaluation of the exotic races under United States conditions, at least in the South. For 216 of the collections evaluated, comparisons were made for time to flowering (50% pollen shed) between the races per se and the means of two test crosses. The mean time to flowering for the races per se was 97.2 days. The earliest collection flowered in 63 days and the latest collection in 131 days, a range of 69 days. When averaged over the two testers, the mean for the 216 test crosses was 74.5 and varied from 61.5 to 84.2 days, a range of only 22.7 days. Although there was essentially no difference between the races per se and the test crosses for the earliest-flowering collections, the latest-flowering collection flowered about 40 days earlier in the test crosses. Most of this change was probably due to the masking of the photoperiodic response by the testers. Those collections that developed slowly under short days were not as greatly affected by test crossing as those for which photoperiod was a primary cause of delayed reproductive development.

Evaluations of yield and other agronomic traits on the test crosses for 601 collections were conducted at six sites in four states in the southern United States. Because the number of collections was too large for evaluation in a single year, a different set of 108 collections (on two testers) was evaluated each year at the six sites. Initial evaluations of diseases and insect pests were conducted over a period of three years, at the rate of 200 collections per year. Because the level of infection or infestation varied from year to year, with the inherent difficulty of making comparison among evaluations conducted in different years, about 10% of the collections (test crosses) exhibiting the highest resistance from each year’s tests were then reevaluated together in a subsequent year. This provided an assessment of the most promising materials under similar conditions.

A listing of data for grain yield, grain moisture, ears per plant and plant height for the 22 highest-yielding collections (test crosses) are presented in Table 4. Because the 601 entries could not be evaluated in a single year, data for grain yield are presented relative to a reference hybrid used in every test. This group of 22 collections originates from nine Latin American countries and includes a wide variety of kernel types including flints, flours, dents and pop-
Table 4. Means of grain yield and three other agronomic traits for the 22 highest-yielding test crosses of exotic collections on two United States single crosses.

<table>
<thead>
<tr>
<th>Race</th>
<th>Collection</th>
<th>Grain yield relative to reference (%)</th>
<th>Moisture content (%)</th>
<th>Ears per plant</th>
<th>Plant height (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lenha</td>
<td>RGS XX</td>
<td>95.2</td>
<td>23.2</td>
<td>1.25</td>
<td>2.93</td>
</tr>
<tr>
<td>Amarillo Salvadoreño²</td>
<td>Bov 806</td>
<td>87.7</td>
<td>23.5</td>
<td>1.37</td>
<td>3.10</td>
</tr>
<tr>
<td>Pororo</td>
<td>Gin I</td>
<td>86.9</td>
<td>21.5</td>
<td>1.84</td>
<td>2.99</td>
</tr>
<tr>
<td>Cateto Nortista</td>
<td>Cub 54</td>
<td>86.2</td>
<td>18.7</td>
<td>1.21</td>
<td>3.18</td>
</tr>
<tr>
<td>Chandelle</td>
<td>Arg II</td>
<td>85.3</td>
<td>11.7</td>
<td>1.33</td>
<td>2.87</td>
</tr>
<tr>
<td>Cateto Sulino</td>
<td>IVC 2</td>
<td>84.8</td>
<td>14.1</td>
<td>1.33</td>
<td>2.67</td>
</tr>
<tr>
<td>St Croix</td>
<td>RGS XVI</td>
<td>84.5</td>
<td>12.8</td>
<td>1.13</td>
<td>3.09</td>
</tr>
<tr>
<td>Semi Dentado Riograndense</td>
<td>RGS XVI</td>
<td>84.3</td>
<td>24.3</td>
<td>1.23</td>
<td>3.07</td>
</tr>
<tr>
<td>Dente Riograndense Liso</td>
<td>RGS VI</td>
<td>84.2</td>
<td>21.7</td>
<td>1.14</td>
<td>3.08</td>
</tr>
<tr>
<td>Canario De Ocho</td>
<td>Urg VI</td>
<td>84.1</td>
<td>13.4</td>
<td>1.18</td>
<td>2.73</td>
</tr>
<tr>
<td>Puya</td>
<td>Vcn</td>
<td>83.8</td>
<td>19.4</td>
<td>1.25</td>
<td>3.08</td>
</tr>
<tr>
<td>Tuscon</td>
<td>Bai III</td>
<td>83.5</td>
<td>21.2</td>
<td>1.17</td>
<td>3.03</td>
</tr>
<tr>
<td>Arizona</td>
<td>Aya 41</td>
<td>83.5</td>
<td>22.1</td>
<td>1.08</td>
<td>3.09</td>
</tr>
<tr>
<td>Denteado Comercial</td>
<td>Chi 326</td>
<td>83.5</td>
<td>18.8</td>
<td>1.28</td>
<td>2.84</td>
</tr>
<tr>
<td>Avati Pichinga Hu</td>
<td>Pag 169</td>
<td>83.0</td>
<td>14.3</td>
<td>1.92</td>
<td>2.72</td>
</tr>
<tr>
<td>Moroti Precoce</td>
<td>Bol I</td>
<td>82.7</td>
<td>18.7</td>
<td>1.34</td>
<td>2.83</td>
</tr>
<tr>
<td>Cristal</td>
<td>MG III</td>
<td>82.5</td>
<td>19.1</td>
<td>1.13</td>
<td>3.02</td>
</tr>
<tr>
<td>Dente Branco</td>
<td>RGS X</td>
<td>82.3</td>
<td>22.7</td>
<td>1.09</td>
<td>3.07</td>
</tr>
<tr>
<td>Cubano Dentado</td>
<td>Bov 585</td>
<td>82.1</td>
<td>17.9</td>
<td>1.21</td>
<td>3.17</td>
</tr>
<tr>
<td>Cateto Sulino</td>
<td>Arg III</td>
<td>81.9</td>
<td>21.8</td>
<td>1.32</td>
<td>2.78</td>
</tr>
<tr>
<td>Cateto Sulino Escuro</td>
<td>Urg V</td>
<td>81.8</td>
<td>17.4</td>
<td>1.38</td>
<td>2.53</td>
</tr>
<tr>
<td>Negro De Tierra Caliente</td>
<td>Gua 159</td>
<td>81.7</td>
<td>13.8</td>
<td>1.19</td>
<td>2.95</td>
</tr>
</tbody>
</table>

1. Reference (Pioneer 3369A) yielded 7.03 Mg ha⁻¹ (0.703 kg m⁻²). Range of grain yield for 601 test crosses was 4.75 to 95.15% of reference.
2. A composite from El Salvador.

corns. Although several of the collections in this group of 22 undoubtedly have been introgressed with United States maize germplasm, many appear to be free from this introgression. The highest-yielding test cross, which yielded 95% of the reference hybrid, was made with Lenha. This collection, from Brazil, appears to be free from United States introgression and has shallow floucy white kernels with a thick but poorly developed cob. However test crosses with Lenha produced an ear that scored higher in overall quality than the reference hybrids used in the evaluations. These data demonstrate that subjective evaluations based on characteristics such as ear and kernel types of races per se may show little relationship with the inherent combining ability in hybrids or test crosses. Data from the entire set of 601 collections (test crosses) have been summarized and are being prepared for publication, probably as a North Carolina Agricultural Research Service Bulletin.

As indicated earlier, one of the problems associated with the use of exotic germplasm is linkages of favorable genes in crosses of exotic with adapted populations. These linkages may not easily be broken and there is considerable circumstantial evidence that restriction to recombination, probably due to cryptic structural differences, may be common in crosses of widely divergent materials. In an effort to measure this effect on recombination, a base population (F1) was synthesized from the Bolivian landrace, Cubano Dentado (Bov 440) and the
Minnesota synthetic, AS-D. Six additional generations (RM1 to RM6) were produced by enforced random mating, using large populations. Analyses of allozyme marker loci in progeny of several selfed plants from each generation (F1, RM3, and RM6) showed considerable heterogeneity for restriction to recombination in all generations. For example, estimates of the recombination fraction for the segment Got 1 – Mdh3 (on chromosome 3L) varied from 0.12 to 0.24 in the F1, from 0.14 to 0.24 in the RM3, and from 0.11 to 0.21 in the RM6. In populations of adapted materials in which there is no reason to suspect recombination restrictions, estimates of this recombination fraction are 0.26.

Use of exotic germplasm in breeding programs

Based on evaluations of the 601 racial collections discussed above, I have chosen 10 promising collections as sources of germplasm for the development of 37 widely divergent breeding populations. The proportion of exotic germplasm in these populations ranges from 25 to 100% and each has been randomly mated for 6 to 8 generations. Several selection procedures, including mass, S1 family, and full-sib family, are being used to improve adaptation as well as yield, disease resistance, and other agronomic traits in some of these populations.

In order to evaluate the heterotic response of these 37 populations with the Stiff Stalk Synthetic and the Lancaster populations, each was crossed reciprocally to two testers: BS13(S)C3, a selected population developed at Iowa State University from the Stiff Stalk Synthetic, and Mo17, a widely used inbred line from the Lancaster group. The crosses were evaluated in three environments in the summer of 1984. Grain yield and plant height for crosses to 10 of the divergent populations and means over crosses to the 37 populations are shown in Table 5. Mean grain yield for the 10 crosses shown all exceeded the yield for the widely grown reference hybrid (B73 × Mo17). Also, comparisons of the reciprocal crosses showed that grain yields for the 10 crosses (Table 5) were usually significantly higher for the exotic cytoplasm than for the tester (United States) cytoplasm. This relationship was also true for the comparison among the means of the 37 crosses. For plant height, comparisons of reciprocal crosses showed that significantly lower plant height was usually associated with the exotic cytoplasm.

Results from this study are significant in that they demonstrate the superior genetic potential of several exotic sources of germplasm. Although some agronomic traits, such as plant height and ear quality, will require considerable improvement before the populations are acceptable in commercial breeding programs for grain yield, the populations may be quite suitable for silage. These results are also significant in that they demonstrate the need to consider the cytoplasm in crosses among widely divergent materials. Such dramatic differences have usually not been found in crosses of more closely related materials and most breeders have assumed that reciprocal differences are not significant. This assumption will have to be reconsidered in the light of these data.

In a similar study to be reported by Gutierrez-Gaitan et al. (1986), 24 improved populations of Mexican germplasm were crossed with two testers, BS13(S)C3 and Lancaster Composite from Iowa State University. These two sets of test crosses were evaluated in eight environments in Mexico and four environments in the western United States Corn Belt. Few of the test crosses yielded more than the four reference hybrids when they were evaluated in the Corn Belt. However both sets of test crosses yielded significantly more than the reference crosses when evaluated in Mexico. Although this germplasm should be useful in Mexican breeding programs, potential use in United States breeding programs appears to be somewhat limited.
Table 5. Grain yield and plant height of F₁'s of exotic populations crossed on two United States testers evaluated in three environments.

<table>
<thead>
<tr>
<th>Population</th>
<th>Proportion germplasm exotic (%)</th>
<th>Tester</th>
<th>Grain yield (Mg ha⁻¹)</th>
<th>Plant height (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>exotic♂</td>
<td>tester♂</td>
</tr>
<tr>
<td>Cubano Dentado (Bov 585)</td>
<td>100</td>
<td>BS13(S)C3</td>
<td>7.79</td>
<td>7.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mo17</td>
<td>7.19</td>
<td>7.01</td>
</tr>
<tr>
<td>Cateto (Ce I)</td>
<td>100</td>
<td>BS13(S)C3</td>
<td>8.93</td>
<td>7.96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mo17</td>
<td>8.20</td>
<td>7.70</td>
</tr>
<tr>
<td>Tuson (Bai III) × Lenha (RGS XX)</td>
<td>100</td>
<td>BS13(S)C3</td>
<td>–</td>
<td>7.59</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mo17</td>
<td>7.25</td>
<td>7.30</td>
</tr>
<tr>
<td>JR10 × (Tuson × Lenha)</td>
<td>50</td>
<td>BS13(S)C3</td>
<td>7.84</td>
<td>8.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mo17</td>
<td>7.78</td>
<td>7.62</td>
</tr>
<tr>
<td>Lenha (RGS XX) × Tuson (Bai III)</td>
<td>100</td>
<td>BS13(S)C3</td>
<td>7.96</td>
<td>8.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mo17</td>
<td>8.03</td>
<td>8.03</td>
</tr>
<tr>
<td>JR10 × (Lenha × Tuson)</td>
<td>50</td>
<td>BS13(S)C3</td>
<td>8.32</td>
<td>7.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mo17</td>
<td>8.21</td>
<td>8.00</td>
</tr>
<tr>
<td>Cristal (MG III) × Arizona (Aya 41)</td>
<td>100</td>
<td>BS13(S)C3</td>
<td>7.45</td>
<td>7.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mo17</td>
<td>8.20</td>
<td>7.99</td>
</tr>
<tr>
<td>IR10 × (Cristal × Arizona)</td>
<td>50</td>
<td>BS13(S)C3</td>
<td>7.81</td>
<td>7.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mo17</td>
<td>7.79</td>
<td>7.64</td>
</tr>
<tr>
<td>Arizona (Aya 41) × Cristal (MG III)</td>
<td>100</td>
<td>BS13(S)C3</td>
<td>8.07</td>
<td>8.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mo17</td>
<td>7.82</td>
<td>7.66</td>
</tr>
<tr>
<td>IR10 × (Arizona × Cristal)</td>
<td>50</td>
<td>BS13(S)C3</td>
<td>8.34</td>
<td>8.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mo17</td>
<td>8.33</td>
<td>7.44</td>
</tr>
<tr>
<td>(Standard errors)</td>
<td></td>
<td></td>
<td>(0.31)</td>
<td>(0.31)</td>
</tr>
<tr>
<td></td>
<td>BS13(S)C3</td>
<td>6.99</td>
<td>6.93</td>
<td>6.96</td>
</tr>
<tr>
<td>------------------------</td>
<td>-----------</td>
<td>------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>Mean of 37 populations</td>
<td>Mo17</td>
<td>6.76</td>
<td>6.51</td>
<td>6.64</td>
</tr>
<tr>
<td></td>
<td>(Standard errors)</td>
<td>(0.05)</td>
<td>(0.05)</td>
<td>(0.11)</td>
</tr>
<tr>
<td>Grand mean</td>
<td></td>
<td>6.88</td>
<td>6.72</td>
<td>6.80</td>
</tr>
<tr>
<td>(Standard errors)</td>
<td></td>
<td>(0.04)</td>
<td>(0.04)</td>
<td>(0.03)</td>
</tr>
<tr>
<td>Reference (B73 × Mo17)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 6. Means and ranges of grain yield and plant height from full-sib families of four exotic × United States populations (256 families per population) evaluated in three environments.

<table>
<thead>
<tr>
<th>Population</th>
<th>Cytoplasm</th>
<th>Grain yield (Mg ha⁻¹) mean</th>
<th>range</th>
<th>Plant height (m) mean</th>
<th>range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lenha (RGS XX) × AS-D</td>
<td>AS-D</td>
<td>5.17</td>
<td>3.42-6.89</td>
<td>2.60</td>
<td>2.03-3.18</td>
</tr>
<tr>
<td></td>
<td>Lenha</td>
<td>5.05</td>
<td>3.62-6.03</td>
<td>2.54</td>
<td>2.16-3.05</td>
</tr>
<tr>
<td>Cateto (Bov 815) × AS-A</td>
<td>AS-A</td>
<td>5.03</td>
<td>3.63-6.70</td>
<td>2.92</td>
<td>2.41-3.43</td>
</tr>
<tr>
<td></td>
<td>Cateto</td>
<td>5.44</td>
<td>3.85-7.25</td>
<td>3.00</td>
<td>2.41-3.43</td>
</tr>
<tr>
<td>Amarillo Salvadoreno × IR10</td>
<td>IR10</td>
<td>6.79</td>
<td>4.69-9.52</td>
<td>3.23</td>
<td>2.79-3.94</td>
</tr>
<tr>
<td></td>
<td>Am Sal</td>
<td>6.61</td>
<td>4.68-8.18</td>
<td>3.39</td>
<td>2.79-3.81</td>
</tr>
<tr>
<td>Camélia (Chi 411) × JR10</td>
<td>JR10</td>
<td>6.47</td>
<td>4.10-8.71</td>
<td>3.12</td>
<td>2.41-3.56</td>
</tr>
<tr>
<td></td>
<td>Camélia</td>
<td>6.51</td>
<td>4.49-8.49</td>
<td>3.13</td>
<td>2.54-3.56</td>
</tr>
<tr>
<td>Reference (B73 × Mo17)</td>
<td></td>
<td>7.30</td>
<td></td>
<td>2.74*</td>
<td></td>
</tr>
</tbody>
</table>

Full-sib family selection is being conducted in several of the widely divergent populations in my program in North Carolina. Overall means and ranges for grain yield and plant height are shown in Table 6 for four of the six population used in this type of selection. To prevent the loss of the exotic cytoplasm, two versions of each population are maintained, one for each of the two cytoplasms involved in the cross. Mean family yields for two of the populations - Amarillo Salvadoreno (a composite from El Salvador) × IR10 (a selected population derived from the North Carolina cultivar, Indian Chief); and Camélia (from Chile) × JR10 (a selected population derived from the North Carolina population, Jarvis Golden Prolific) – approached the yield of the reference cross (B73 × Mo17). Numerous families in each of these populations yielded more than the reference cross. However, traits such as standability, plant height and ear height will require considerable improvement before these populations are acceptable to commercial maize breeders.

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Use of exotic maize material and building up of broadly based populations for European breeders

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Summary

The definition of exotic variability is broadened to local unadapted material. Besides examples of use of exotic maize to fulfilling specific disease and pest resistance, climatic adaptation or silage value objectives, we emphasize opportunities to build new heterosis patterns and to breed better general combining abilities. The first difficulty is to manage numerous local populations. The second is to screen unadapted material for economic value. After reviewing the pioneer work of CIMMYT for the tropical areas, some results of early conversions and tropical x temperate crosses are examined. The French cooperative programme of maize genetic resources management is then described. About 1500 populations have been introduced, multiplied and evaluated (morphological and agronomic characters per se, general and specific combining ability with 3 testers for grain and silage value). Conversely about 75 broadly based pools will be built using criteria of combining ability. Some breeding populations will be made as well as basic investigations about the relation between population characters and heterosis.

Descriptors: maize, Zea mays L., exotic germplasm, heterosis, combining ability

Broadening the definition of exotic variability to local unadapted material

When we call a material exotic, in the case of maize, an allogamous cereal bred through extensive use of heterosis, we need a working definition. Precisely speaking, exotic maize is germplasm from another area (Lonnquist, 1974). In Europe, maize has been grown for only a few centuries, and it is difficult to draw a limit that could define exotic maize by date of introduction or by amount of combination with ‘local’ material.

For the breeder, a precise definition is not important. The main problem with ‘exotic’ or ‘local’ variability is good management of genetic resources. The main maize-producing countries have extensive breeding programmes. There is little doubt that any material will be maintained and used if it is well adapted to local environmental conditions and economic needs.

Inversely, there is a risk that unadapted material from any origin will not be worked out; its relative value will be lower when genetic advances can be obtained through the main flow of breeding; finally it could be lost or appear totally obsolete and useless. This occurs drastically with the rate of replacement of local European traditional germplasm by material from the North American Corn Belt, as is observed in the commercial cultivars as well as breeding programmes.

So we propose that for working purposes the concept of exotic maize germplasm be broadened to ‘exotic or unadapted germplasm’. Another illustration of this concept would be to consider jointly spatial exotism and time exotism (old populations). Both are generally unadapted.
However ‘spatially exotic’ maizes are sometimes more adapted than local races. It could be argued in order to illustrate this paradox that except for tropical American maizes (dominated by ‘indigenous’ Mexican Tuxpeño and South American Cateto racial complexes), all maize cultivars have arisen from foreign races for example North American Corn Belt, through the hybridization between New England Flint and Southern Dent racial complexes during the European colonization (Gerrish, 1983).

In Europe, hybrids from early maturity groups (FAO scale <350) are normally flint-dent hybrids obtained by crossing United States Corn Belt Dent with European flint. Among the European flints, the genetic base used is very close. Derieux (1979) shows that inbred line F2 was used in France in 1977 in 87% of the seeds produced in the early maturity group (FAO scale <320). The situation is probably not better today.

What could be gathered from exotic maize?

Disease and pest resistance There are numerous reports outside Europe of recovery from exotic maize of resistance factors to various diseases and pests. However it is not particularly useful to review those results because Europe is less affected than other intensive maize-growing areas (southern United States Corn Belt, central China) by leaf diseases. The resistance to other diseases related to the physiological status of the plant (for example Fusarium sp. related to senescence, Barrière et al., 1981) is difficult to define since we have no clear-cut mechanism of resistance.

Resistance to European corn borer (Ostrinia nubilalis) is of more concern to us since the infestation is very heavy under some conditions, making chemical control necessary. Moreover the resistance of most European lines and populations is low. Hence it would be necessary to obtain better resistance associated with earliness. Information and resistant germplasm is actively exchanged by the International Working Group on Ostrinia, an informal association including several European countries. Good sources of resistance to different types of corn borer attacks are present in material from the North American Corn Belt and need to be incorporated into early FAO 300 European cultivars (Kaan et al., 1983). We are, however, not yet sure that resistance alone could completely overcome corn borer attacks and avoid the need for chemical control. We have a good hope that a range of resistance from exotics can be incorporated in European maize.

Broader climatic adaptation Temperature is clearly a limiting factor to the use of late and more productive maize especially in northern Europe (Derieux & Bonhomme, 1982). Moreover cool conditions often prevail in spring in north western Europe. Moisture stress is regularly observed in central, eastern and southern Europe.

Unadapted local European populations were clearly efficient in breeding early varieties tolerant to cool conditions by combination with material from the North American Corn Belt more adapted to modern growing conditions: i.e. high yielding, lodging resistant, tolerant to high density and fertilizer rates (Gallais, 1983). Central European local populations were conversely used by Rumanian breeders for drought tolerance.

New prospects in breeding for tolerance to low temperature arise by the observation of tropical highland populations under such conditions in Europe and New Zealand. Emergence (Eagles, 1982), dry weight of seedlings (Miedema, 1979), rate of leaf emergence (Vincourt, 1983), autotrophic growth (Hardacre & Eagles, 1980) at low temperatures can be improved by using highlands pools, especially from CIMMYT. Cold tolerance is of importance for north-
ern Europe. The maize plant must intercept as much solar radiation as possible during spring and early summer, before solar radiation decreases.

**Fulfilling new purposes** Future silage maize cultivars could be morphologically and genetically very different from present grain types. Production of dry matter from whole plant of exotic or primitive maize type such as ear-prolific or *Teosinte* tillering types is impressive (Barrière et al., 1984). Exotic and unadapted material are often characterized by a low grain-harvest index, which is not detrimental for silage production.

The most important result of maize breeding, as for other cereals, has been the increase of harvest index. For silage utilization, the harvest index can be lower if total dry matter production is high. In this way, the exotic material and such characteristics as prolificacy and tillering will be easier to use than for grain production.

**New patterns of heterosis** There is striking evidence and numerous reports about the use of heterosis between populations or lines from very different areas, for instance lines issued by INRA from the French population Lacaune × North American material in Europe, North American × Eto ‘coastal flint’ population or derivatives in the United States, North American × Cateto Brazilian populations in the United States, North American × Argentine ‘Colorado’ populations or derivative in the United States, Europe and Argentina, French populations derivatives × Argentine ‘Colorado’, in Europe and Argentina, Eto derivative × Mexican Tuxpeño in Brazil (Lascols, 1959; Gerrish, 1983; Oyervides-Garcia et al., 1985; Miranda Filho & Vencovsky, 1984). Our experience about genetic distances measured through analysis of principal components of specific combining ability in inbred dialles shows that spatial relationships are complex and that many cases of specific combining ability should be considered.

This situation contrasts with other cereals like wheat, for which heterosis patterns need to be defined in order to promote the use of hybrid combinations. There are cases where heterosis is used between populations from the same racial complex: North American Reid (Stiff stalk) × North American Lancaster or even between lines from the same population (B73 × B37). Building new heterosis patterns could be the most rapid way to utilize exotic or unadapted material. Conversely, the study of combining ability of unadapted × adapted material is much easier than the study of direct value of the unadapted material and could be fitted to ordinary experimental hybrid value estimations.

**Fixation of heterosis into new racial complexes through recombination** We have at least one very good example of a new racial complex, North American Corn Belt dent originating from the merging of Southern dent and New England flint. The success and adaptability of this material has been outstanding in many countries and could be due to the fixation of favourable alleles after recombination from the preceding races leading to homozygosis for factors formerly considered in heterosis components. Hence it should be useful to try to incorporate heterosis components in ‘superpopulations’ merging races showing mutual effects of specific combining ability.

**Obstacles to the use of exotic and unadapted populations**

**Difficulties in the management of broad-base variability** The management of world maize genetic variability is evidently an enormous job, which necessitates a large amount of international cooperation. The urgency to maintain a specific programme integrating unadapted and
exotic material is not unanimously accepted.

Hallauer (1980) observed that the potential of exotic germplasm had been advocated for over 25 years in the United States but conversely the genetic basis of applied breeding was becoming more restricted. One of the explanations of this contradiction could be that the racial complex of the North American Corn Belt is highly adapted and broad-based and that much could be done using it. This situation is different for European breeders who do not generally possess such adapted variability. Once the decision is taken to start a programme to utilize genetic resources, the first problem is a survey of representative types, preservation and increase of such unadapted populations. A lot of the national genetic resources are not easily to hand for the foreign breeder except through the international programmes of IBPGR and CIMMYT. The second difficulty is the number of accessions. More than 20 000 populations are certainly maintained around the world.

The description and characterization of this material for eventual grouping and management could be theoretically based upon selection by neutral molecular characters such as isozyme or denatured protein two-dimensional electropherogram (Zivy et al., 1983). These methods are presently much too expensive to be generalized and could be only exploratory for specific cases. Local populations could alternatively be characterized by geographic origin. This is easy, and sometimes useful as we know that tropical material are always unadapted. However, intensive germplasm exchange between maize-growing areas could cause duplication if the same population is used by different countries.

Botanical characters and particularly ear and kernel traits are interesting, useful and easy to classify (cob dimensions, number of rows, kernel colour, texture and dimensions). They were particularly used to classify European populations (Brandolini, 1970). However, they have often no direct economic interest. Agronomic traits such as yield, earliness, resistance to root, stalk and ear rot are more evidently useful (Kaan et al., 1983) but often not very heritable. Moreover the inbreeding level could fluctuate between populations and make comparisons uninteresting, especially for yield-related characters. Top cross tests with locally adapted testers would be an efficient manner to screen more genotypes, without inbreeding bias, and to obtain information about general or specific combining ability for economically interesting characteristics.

**Difficulties with tropical material** Apart from the susceptibility to maize smut (*Ustilago maydis*), the main problem is the reaction to longer days, resulting in increased growth of leaves and in a delay in flowering and in maturity, often associated with root lodging. This reaction is rather general. It is impossible to evaluate and even to multiply these populations in Europe. First generation hybrids with temperate genotypes cannot be usefully observed in Europe and even in the United States Corn Belt (Oyervides-Garcia et al., 1983). Experiments by Spencer (1975; 1980) and Russell & Stuber (1983) suggest there is continuous variation in this reaction, which is not directly related to earliness under long or short days. Moreover, like temperate local populations, tropical material may or may not be equally adapted to modern maize-growing conditions but this adaptation is generally masked by daylength reaction. The only way to use this tropical material is tedious breeding work of conversion, in order to obtain long-day tolerant types.

**Experience about exotic germplasm management**

**CIMMYT example** This organisation has probably the best structured 'back-up' unit. A
A detailed description of its work has been published by Vasal et al. (1982). About 12,000 accessions are maintained and evaluated. They are aggregated to the 29 pools created after examination of their performance then the performance of the crosses of the introductions with the corresponding pools. The pools are defined in relation to climatic adaptation, maturity, grain colour and texture. Each pool is maintained and improved through a half-sib recurrent breeding system. Promising accessions are continuously added as female rows. The pools are bred in Mexico, one generation per year at two places. In the next step, the best half-sib families are introduced progressively into the corresponding populations, which are bred in an international progeny-testing full-sib recurrent system. The main emphasis of this fruitful programme is, however, limited to tropical and subtropical developing countries. Hence CIMMYT material is not of direct use for Europe. Another characteristic of this programme is that specific combining abilities are not evaluated.

**Early conversions for northern temperate regions**  This is a familiar process for European breeders and was illustrated by the spectacular conversion by Dr Rinke of B14 line adapted to the central Corn Belt into A632 and other lines extensively used in the northern United States Corn Belt, Europe and Canada. Much of that typical use of unadapted material is so common that one hardly considers to mention it. However this is somewhat dangerous. Even if the success of such materials allows some European countries to obtain yields comparable to the best North American Corn Belt and to subtropical areas, we are dependent on the availability of the best foreign lines and this could cause many difficulties when these lines are proprietary material. Alternative ways are evidently to use or build synthetic populations.

**Temperate conversions of tropical material**  Up to now, the evidence is scattered and unconvincing about the way to manipulate and screen material of tropical origin. Hallauer & Sears (1972) compared two methods of improving tropical Eto synthetic. Mild mass selection could me more efficient than recombination with Corn Belt material. Other conversions of tropical populations were achieved by Gerrish (1983) and many other breeders around the temperate zone. A review presented at a meeting of North American public breeders (Compton et al., 1977) indicates that 44 populations of partially or completely tropical origin were actively bred from a total of 246 populations.

Our experience with tropical material is rather limited. We built two synthetics, FS16 and FS17. FS16 originated for 25% from West African IRAT composites and 75% from material adapted to southern France. There was certainly a considerable amount of breeding for earliness and smut resistance, but FS16 kept some unfavourable ear characters of tropical parents (small cobs, small flint kernels). The synthetic FS17 originated from Caribbean local populations as male parents and material adapted to southern France as female (Texas cytoplasm). We bred for earliness and for resistance to Helminthosporium maydis race T. The plant and ear appearance are quite good but this material is rather late and could interest only southern Europe directly. This experience could be fruitful in warning us against spending too much time converting tropical low-yielding material into a temperate version. The modern CIMMYT tropical populations with a better harvest index could be interesting. Moreover, crossing tropical long-day-susceptible material with adapted material could result in populations of intermediate earliness and not well adapted. This would favour tropical crosses with extra early types to incorporate adequate earliness.
The French programme for management of genetic resources in maize

Participants As in any country where active maize breeding is practised, there have been numerous programmes to maintain and use exotic or unadapted material. However about ten years ago, it became clear that a cooperative venture could give more opportunities to manage a broad-based population efficiently. After some years of consideration and discussion, about 17 private or cooperative breeders and the Institut National de la Recherche Agronomique (INRA) started a five-year programme in 1983, to be prolonged for another five years. This programme is conversely financially helped by government ministries and by the French maize-producers’ union (AGPM).

Origin of accessions The programme includes management of broad-based populations and some initial improvement. In contrast with the emphasis generally given in Europe to narrowly based crosses for line-improvement programmes, only populations ('populations sources') are aggregated into manageable pools. The 'populations sources' are local, exotic, or synthetic populations. We are evaluating about 1 500 populations. We emphasize European local populations. The first step is to introduce, increase and evaluate about 350 accessions each year.

Evaluation Evaluation of temperate populations is essentially:
- An evaluation as such in two stations with individual or plot data on easily observable traits of agronomic or morphological interest. Yield data are only observed to elaborate new methods of selection.
- A test for combining ability with the 3 best available testers. Five earliness groups are considered. Triple lattices are tested at two sites for grain value (on 3 testers) and silage value (on 2 testers).
- About 50 trials are run each year.

Building up broad-based pools The second step is the building of pools. According to results of tests for combining ability in the previous year, the best populations for some criteria are aggregated into pools using isolation plots. For example this year, we are starting 15 pools, of which 6 use grain index criteria, 5 use silage index criteria and 4 are tropical-temperate pools. Other criteria, such as earliness, and specific or general combining ability, are combined with the preceding ones. Direct population evaluation per se is not used for the building up of pools. If we maintain the same rate of pool building, we will have about 75 broad-based populations, which may or may not be merged before starting a population-improvement program.

Results to be obtained Any result or information about this programme is currently integrated in a relational data base and the cooperators can ask any question about any material. The material could be used directly for its specific value in an inbred development by a participant. The pools created could equally be used in this way. However after the pools are built up, the logical step will then be development of a pool-improvement program.

Broad-based improved pools could be one on the best possible sources of germplasm for inbred development. Two main approaches are being investigated:
- mild mass or ear-to-row selection
- some system of reciprocal recurrent selection.
The extent of the second approach will depend on the performance of the pools; if it is good, more emphasis could be given to reciprocal recurrent selection.

**Tropical × temperate material** Our investigations will be limited because evaluation is not possible. Hence it is difficult to screen tropical populations for merging in tropical-temperate pools. However, we are starting to cross about 200 tropical populations with a very early broad synthetic. Pools and populations from CIMMYT will also be crossed with the synthetic. The first crosses used tropical material as female so as to save possible exotic cytoplasmic variability. They were sibbed for two generations and bred for 'normal' earliness and smut resistance. This material now seems rather promising and was merged into 4 pools based arbitrarily upon geographic origin and endosperm texture:
- Caribbean Flint
- African Flint
- Mexican, Southern and South African Dent
- Andean Flour.

However, we intend to backcross these pools to tropical populations in order to recover temperate adapted versions of tropical germplasm.

**Basic investigations** These developments are only a start. In short, this project has proposals to evaluate the relations between per se agronomic, morphological and in some cases molecular (isozyme) and combining ability (SCA and GCA) population characteristics. A better understanding and control of heterosis is anticipated. This French program could appear ambitious and costly. Its results cannot be appreciated now and they will not necessarily be published by the breeders. However, we already have such interesting achievements as:
- active cooperation between private and public breeders
- avoidance of duplication of work in this breeding area.

**Conclusions**

The breeder using exotic material must keep in mind some points:
- it is a long term work
- the objective must be well defined
- the breeding methods must be adapted to have a chance of breaking the blocks of genes by recombination
- there is an antagonism between improvement, adaptation and genetic variability
- cytoplasmic variability must be preserved.

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Cold tolerance
Chilling stress in maize

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Summary

The effects of chilling stress can be divided into survival and avoidance of damages at about 0-6 °C and into maintenance of development at about 6-15 °C. The effect of a certain temperature depends on its duration, diurnal variation and history, as well as on constitution, stage of development and genotype of the plant. The main cause of severe chilling strain seems to be the phase transition of membrane lipids, which is terminated in chilling susceptible species below 8-12 °C. Water balance becomes important at low temperatures. It might be controlled by the phytohormone abscisic acid. Mild chilling stress can independently affect heterotrophic and autotrophic processes. Chilling-tolerant genotypes maintain a coordinated development of leaf and root area at mild stress. Leaf expansion is controlled by the temperature of the shoot apex. With mild chilling, efficient growth depends on efficient carbohydrate utilization whereas stable rates of photosynthesis depend on stable activity of photosynthetic enzymes. With these different causes for chilling strain it is difficult to cover all chilling effects with one method of selection.

Descriptors: maize, Zea mays L., chilling stress

Introduction

A crop will bring highest yields with lowest risk of failure if it is cultivated as close as possible to the specific temperature optima for each of its developmental stages. For several, mainly agronomic, reasons, maize cultivation has been extended to areas that cannot fulfil these conditions. Because of a relatively long period of growth, temperatures are often far below optimum at the beginning and the end. A negative correlation between yield and duration of the growing season requires a compromise between the risk of yield failure and the acceptable yield. In order to minimize the risk, breeders select for chilling tolerance. But progress is often slow as a genetic background for real chilling tolerance is missing and knowledge of the physiological and biochemical causes of chilling susceptibility is still insufficient. Adaptation to low night temperatures and to wide diurnal fluctuations in temperature may be found in some exotic germplasm from tropical highlands. But climatic conditions there do not closely resemble situations of chilling stress in North-West Europe.

The term chilling tolerance is used for different reactions of the plant towards temperature. It can comprise survival of severe chilling stress (about 0-6 °C) and maintenance of growth processes with mild chilling stress (about 6-15 °C). It cannot be a realistic goal to look for one simple method of selection to cover all aspects of chilling tolerance. Some chosen examples will be used to explain the different reactions of maize genotypes towards severe and mild chilling stress. Details are contained in three recent reviews (Miedema, 1982; Crèvecœur & Ledent, 1984; Stamp, 1984).
Severe chilling stress

Severe chilling stress can damage or kill the plant within hours or a few days. Temperatures below the minimum for growth are mostly responsible for this kind of strain. It is difficult to foresee when plants will be subject to severe or mild chilling strain. This depends on the temperature as well as on the plant.

The most important cause of damage by severe chilling seems to be a phase change of membrane lipids. In chilling sensitive crops, membrane lipids undergo a transition from liquid-crystalline to solid gel phase, which is terminated below 8-12 °C. A higher permeability of the membrane with severe chilling may directly injure and kill the cell because of an efflux of ions and metabolites. Indirectly the higher permeability together with a higher activation energy of membrane-bound enzymes may lead to accumulation of toxic metabolites.

Several years ago, it was general belief that the phase transition takes place at an exactly definable temperature. Nowadays the opinion is held that the phase transition is a gradual process that starts in certain areas of the membrane when the temperature is lowered from about 25-30 °C to 8-12 °C (Forsyth et al., 1977; Bagnall & Wolfe, 1978). These new findings make it impossible to devise a selection method based on the temperature at which phase transition takes place.

About ten years ago, the essential difference between chilling-tolerant and chilling-sensitive species was seen in a higher content of unsaturated fatty acids in membrane lipids. Accordingly, the conditioning of sensitive species towards low temperature was explained by an increase of unsaturated fatty acids in membrane lipids (Lyons, 1973; Raison, 1973; Lyons & Breidenbach, 1979). If this had been so, a selection method might have been based on analysis of membrane lipids for fatty acids by gas chromatography. But general consensus was broken some years ago when several investigators refuted any relationship between tolerance to severe chilling and fatty acid composition of membrane lipids (Raison & Chapman, 1976; Wilson, 1976; Hannon & Raison, 1979). In the last two years, some scientists returned to the idea that fatty acid composition is involved in chilling tolerance when they thoroughly analysed different species of membrane lipids (Murata & Yamada, 1983; Christiansen, 1984). One component of the chloroplast membrane, the phospholipid phosphatidyl glycerol, has a low content of unsaturated fatty acids in chilling-sensitive plants. In these plants, phase transition of this lipid starts at room temperature.

I have presented these new findings to show that old simple models sometimes have to be replaced by complicated new ones that do not allow the development of direct selection methods. Indirect selection methods may be found yet. For instance, a method based on the decrease in chlorophyll fluorescence with severe chilling stress might become suitable for practical purposes (Havaux & Lannoye, 1984).

Other, probably less important causes of damage by severe chilling stress are structural changes in some enzymes (Chollet & Anderson, 1977; Shirahashi et al., 1978).

Mild chilling stress

Below 15 °C, growth processes are slow in maize. They come to a standstill at about 6-10 °C. This temperature range represents mild chilling for maize plants. Levitt (1980) calls it an 'elastic strain' as most plants resume normal development when the stress is removed.

Contrary to severe chilling, mild chilling induces complex reactions, which often seem to be independent of one another. For instance during the early seedling stage, we can distinguish
effects on heterotrophic development, like utilization of kernel reserves, and growth of roots and leaves from effects on autotrophic processes like photosynthesis and nutrient uptake.

The genotypic variability for germination at low temperature is quite appreciable when temperatures are above 10 °C. Below that limit, the germination process is very slow and genotypic differences are small (Crèvecoeur & Ledent, 1984; Stamp, 1984), perhaps through phase transition of membrane lipids. The question of kernel reserves has been often raised in connection with genotypic differences. Investigations of Christeller (1984) have clearly proved that growth rate is dependent only on the embryo.

The influence of temperature of the root medium has been studied mostly for effects on the shoot. Reactions of the roots themselves have been largely neglected. Furthermore, the majority of these investigations have been done in hydroponic culture, from which it is difficult to generalize to root development under field conditions.

Already 50 years ago, Smith (1934) detected inadequate phosphorus uptake at low temperature in relation to poor root branching. Progress on this field has not been very fast; much is still guesswork. Grasses normally expend the highest proportion of dry matter on root growth at unfavourable temperatures. But below 15 °C, maize seedlings lose the ability to expend dry matter on development of the root in favour of the shoot and the shoot-root ratio increases again. A similar reaction has recently been reported for cereals from temperate regions when grown at 5 °C (Abbas Al-Ani & Hay, 1983).

Information about area ratio of leaf to root is scarce although its physiological relevance is much greater than that of mass ratio of dry matter accumulation in shoot to root. At the same physiological age, favourable temperature and uniform conditions for growth, the area ratio of leaf to root seems to be almost constant for a wide range of maize genotypes (Fig. 1). The root type did not matter for this ratio. Edo, for instance, represented an intensive type with fast development of laterals, Huanquero an extensive type with fast development of main roots. The area ratio of leaf to root is affected by low temperature in accordance with the chilling tolerance of a genotype. Very stable ratios were observed for some top crosses between European hybrids and highland races.

![Percentage of area ratio of leaf to root at 3-leaf stage. 15 °C (white) and 25 °C (grey). Data from Stamp (1983).](image)
Besides effects on root development, low temperatures can directly influence the function of the root. The most cited example and the most controversial results can be found for phosphorus uptake with mild chilling stress (Knoll et al., 1964; Ketcheson, 1966; 1968; 1970; Patterson et al., 1972; Ferguson & Clarkson, 1975). Many of these investigations hardly took into account that all physiological processes are slowed down by low temperature. When plants were compared at the same physiological age, the 4-leaf stage, the accumulation of phosphorus and potassium had been much less hampered by low temperature than growth itself, regardless of a high or low supply of nutrient (Fig. 2). Therefore it seems unlikely that inability to take up these nutrients limits growth of seedlings. Sutton (1969) concluded in an early review that low temperatures might reduce the availability of soil phosphate to the plants. In cold soils, the poor development of root area impedes phosphorus uptake too.

Shoot growth and leaf area development are both severely affected by low temperature. Some half of the long-term effect of temperature on growth is caused by its direct influence on processes like photosynthesis, uptake of water and nutrients. The other half of the long-term effect is caused by the indirect influence of temperature on processes through changes in the morphology of root and shoot (Brouwer et al., 1973). In young maize seedlings, shoot growth is closely linked to leaf expansion. Nevertheless, leaf expansion reacts much sooner to a drop of temperature than accumulation of dry matter. This differential chilling effect has been explained by the high temperature sensitivity of the shoot apex (Watts, 1972; Kleinendorst, 1975; Barlow et al., 1977). As the shoot apex is situated below the surface until the 8-leaf stage (Brouwer et al. 1973), the temperature of the surface soil influences leaf expansion.

In the field, short spells of cool and warm weather often alternate. A fast recovery and an efficient utilization of favourable temperatures might be as decisive for chilling tolerance of a genotype as the ability to maintain growth during chilling stress. Genotypes different in chilling tolerance were grown either at constant 14 °C or alternately three days at 18 °C and three days at 10 °C (Stamp, 1984). All genotypes considerably profited from alternating temperatures. Marked differences in the resumption of growth were not observed.

Though the cessation of growth at low temperatures seems to be mediated by the shoot apex, the rate of dry matter accumulation with mild chilling stress might be controlled by the efficiency of carbohydrate utilization. When six inbred lines were grown at 14 °C, the shoot dry weight and phosphofructokinase activity were closely related (Fig. 3). Phosphofructokinase
has an important function in carbohydrate utilization as its activity regulates cytoplasmic glycolysis.

How does the plant coordinate its reactions towards mild chilling stress? At the moment, no satisfactory answer can be given. There are indications that phytohormones are involved. For instance, the translocation of growth inhibitors from the root increases with chilling (Atkin et al., 1973). This process may be associated with economical consumption of water with chilling as the aperture of the stomata is closely correlated with the content of abscisic acid, a growth inhibitor (Raschke, 1975). Mustardy et al., (1982) showed that a chilling susceptible variety lost control of stomatal opening under stress. According to Wilson (1976), the hardening of chilling sensitive plants predominantly causes avoidance of undue water stress.

As early growth of seedlings is sustained to a large extent heterotrophically by kernel reserves, no close correlation between growth and photosynthetic traits can be expected before the 3-leaf stage. However in closely related maize genotypes of slow or fast growth at 14 °C, the latter group had much higher activity of photosynthetic enzymes such as nicotinamide adenine dinucleotide phosphate (NADP) malate dehydrogenase, though chlorophyll content was almost identical for the two groups (Stamp, 1984).

Chlorophyll content is rather insensitive to chilling stress if the leaves have developed fully at favourable temperatures (Alberda, 1969; Stamp, 1984). However the activity of the NADP malate dehydrogenase is reduced in chilling susceptible genotypes by short-term as well as long-term chilling stress (Fig. 4). This might explain a similar sensitivity of the photosynthetic rate in these genotypes.

Low temperatures cause chlorotic leaves in the field. McWilliam & Naylor (1967) explained the inability of maize seedlings to accumulate chlorophyll under moderately cool conditions and high light intensity by the photo-oxidation of chlorophyll before incorporation in photosensitive membrane configurations. Certain carotenoids can protect against this detrimental photosensitization (Krinsky, 1978). Comparison between North-West European and subtropical inbred lines showed that adapted lines reacted towards low temperature with an increased content of carotenoids (Fig. 5). Such a reaction was not observed in subtropical lines.

Chilling strain has several causes that act independently on a certain genotype. So it will be difficult to find one selection method that covers all effects of chilling.
Fig. 4. Assimilation rate expressed as mass rate of CO₂ divided by mass of fresh matter (A) and content of nicotinamide adenine dinucleotide phosphate malate dehydrogenase in fresh matter (B) of second leaves of two inbred lines. Seedlings were grown either at 24 °C/22 °C (grey) or at 14 °C/12 °C (white) until full expansion of the second leaf (time zero). Thereafter the temperature was changed from 24 °C/22 °C to 14 °C/12 °C or vice versa. Data from Stamp (1984).

Fig. 5. Content of carotenoids in fresh matter of fully expanded second leaves of maize seedlings. Means are presented for three adapted European inbred lines (●) and for three subtropical inbred lines (○) grown at four temperatures. Data from Stamp et al. (1983).
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Effect of low temperature (10 °C) on growth, mitotic index and cell ultrastructure of maize leaves

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Summary

Young maize seedlings grown at 20 °C were transferred to 10 °C for increasing periods of time. In mature leaves, changes in ultrastructure became apparent after chilling for as long as three weeks. The nucleus was less affected than cytoplasmic organelles. In the youngest expanding leaves, leaf extension and elongation in the basal region ceased at the onset of chilling. Mitotic index in their basal region sharply decreased within a few hours following transfer of plants to 10 °C. No ultrastructural modifications were noticeable in meristematic cells after three days of chilling.

Descriptors: maize, Zea mays L., chilling stress, low temperature, growth, mitotic index, cell ultrastructure

Introduction

Maize is one of the plants generally recognized as cold-sensitive. In cool climates, low temperature in spring is one of the major environmental factors limiting germination and early growth of maize in the field. Growth of young maize at low temperatures seems to be more limited by the action of cold on leaf growth than by photosynthesis (Miedema & Sinnaeve, 1980). The effects of low temperature on leaf extension have been examined in a few studies (Miedema, 1982; Crèvecoeur & Ledent, 1984). Direct effects of temperature on the leaf meristematic region have been suggested (Watts, 1972). We have investigated the effects of the transfer of young maize seedlings from 20 °C to 10 °C on ultrastructure of a mature leaf, growth and ultrastructure of the youngest expanding leaf, and mitotic index of its basal region.

Material and methods

Plant material  Maize (Zea mays L. cv. Beaupré) was grown from seed in soil in a controlled environment chamber (temperature 20-22 °C; photosynthetic irradiance 17 W m$^{-2}$; relative humidity 75%) with daylength 12 h until complete expansion of the three first leaves (about three weeks). Plants were then transferred to a chilling room at a constant day and night temperature of 10 °C for increasing periods varying from a few hours to three weeks (6 h; 1, 2, 3, 7, 14 and 21 d). Control plants were maintained at 22 °C.

Leaf growth measurements  The method of Michelena & Boyer (1982) was used to study the effect of chilling on elongation of the youngest expanding leaves (fourth and fifth leaves), elongation of the entire leaf and elongation profile along the leaf.
Histology  The basal region of the youngest expanding leaf of plants grown continuously at 20 °C and of plants transferred from 20 °C to 10 °C for 6 h or 3 d were fixed in Navachine. After thorough washing in distilled water, the specimens were dehydrated in graded alcohol and embedded in paraffin. Longitudinal sections (7 μm thick) were stained with haematoxylin. They were examined under a light microscope with a × 100 immersion objective and the mitotic index was estimated.

Electron microscopy  Small pieces of leaf tissue were taken from the middle region of the youngest mature leaf (third leaf) and from the meristematic region of the youngest expanding leaf (fifth leaf) of control and chilled plants. They were fixed in 4% glutaraldehyde buffered with sodium cacodylate (0.2 mol/L; pH 7). Specimens were then washed in cacodylate buffer, fixed in 2% OsO₄, washed in distilled water, dehydrated in a graded ethanol series and embedded in an Epon mixture. Ultrathin sections (60 nm thick) were cut with a Porter Blum MT2 ultramicrotome, then stained with saturated aqueous uranyl acetate and Reynolds lead citrate. Electron micrographs were made with a Siemens Elmiskop 101 microscope at 80 kV.

Results

The extension rate of the two youngest expanding leaves of plants transferred from 20 °C to 10 °C completely stopped during the first hours of chilling (Table 1). It recovered slightly for longer chilling exposure but extension proceeded very slowly compared to control leaves. Similar observations were made for elongation profile along the leaf.

In plants maintained at 10 °C for long periods the rate of growth in length of expanding leaves remained about 0.9 cm/d during the first two weeks. It decreased to 0.5 cm/d during the third week of chilling. Recovery of leaf extension after return to 20 °C proceeded quickly after chilling treatments of 6 h or 3 d. For longer periods at 10 °C, the longer the chilling period, the slower the recovery.

The proportion of mitotic cells (mitotic index) in the basal region of the youngest expanding leaf decreased from 10 to 2.4% 6 h after transfer of plants from 20 to 10 °C (Table 2). It was unchanged after 3 days of chilling.

Ultrastructure of mature leaves  Figure 1 shows cells from a mature leaf of maize plants grown at 20 °C, with the two well known types of chloroplasts. In mesophyll cells (Figure 1A), the chloroplasts showed many well developed stack grana and many stroma lamellae interconnections. Bundle sheath cells contained larger chloroplasts characterized by extensive stroma

Table 1. Effect of transfer of maize seedlings from 20 °C to 10 °C on leaf elongation.

<table>
<thead>
<tr>
<th>Time after transfer (h)</th>
<th>Av. increase in length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf 4 (at 20 °C)</td>
</tr>
<tr>
<td>4</td>
<td>0.57</td>
</tr>
<tr>
<td>7</td>
<td>0.88</td>
</tr>
<tr>
<td>24</td>
<td>3.60</td>
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<tr>
<td>48</td>
<td>7.30</td>
</tr>
<tr>
<td>72</td>
<td>10.95</td>
</tr>
</tbody>
</table>

52
Table 2. Effect of keeping plants at 10 °C for 6 h and 3 d on mitotic index (%) of the basal region of an expanding leaf.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (20 °C)</td>
<td>10.10</td>
</tr>
<tr>
<td>6 h at 10 °C</td>
<td>2.38</td>
</tr>
<tr>
<td>3 d at 10 °C</td>
<td>2.70</td>
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</tbody>
</table>

lamellae and starch grains (Figure 1B). Both types of cells were highly vacuolate and their plastids pressed against the cell wall.

There was no obvious ultrastructural modification in mature leaves of plants kept at 10 °C for a few hours to two weeks. Ultrastructural alterations were noted after keeping cool for three weeks (Figure 2). In bundle-sheath cells, the chloroplasts lost their elongated form to take a very irregular form (Figure 2B). They still contained starch grains. They showed many long non-appressed membranes and developed myelinic-like membranes in their centre. In mesophyll cells (Figure 2A), plastids still contained many stacked grana. They showed modifications in their membrane organization: swelling of unappressed lamellae; dissociation of some thylakoids; appearance of peculiar membrane structure (arrow). Droplets were noted that stained in osmic acid. Both types of chloroplastic cells appeared less vacuolate than control cells and were very poor in ribosomes and mitochondria. No noticeable change was apparent in the nucleus. In some cells, ruptures were seen in some membranes.

Fig. 1. Electron micrographs of chloroplasts from a mature leaf (third leaf). A. Mesophyll. B. Bundle sheath. ×2 340 (1); ×11 200 (1A); ×11 000 (1B)
Ultrastructure of expanding leaves  Cells of the basal meristematic region from leaves of plants grown at 20 °C contained small plastids. Their internal membrane system consisted of a few thylakoids; one or two large starch granules occupied most of the stroma (Fig. 3). No difference was detected in these cells between the mesophyll and the bundle-sheath plastids.
In the nucleus, the nucleolus was predominantly fibrillar with a few granules at its periphery; it showed condensation of its associated chromatin into an organizer region (NOR). A few areas of dense chromatin were seen in the extranucleolar region of the nucleus (arrow). Mitochondria were generally circular and had a few cristae. No noticeable modification was noted in the fine structure of these meristematic cells after 3 days at 10 °C.

Conclusions

In mature leaves, only very long chilling periods altered ultrastructure. The absence of early effects on chloroplasts was not unexpected, since irradiance was low and the severity of damage to chloroplasts was known to increase with irradiance (Miedema, 1982; Crèvecoeur & Ledent, 1984). In expanding leaves, growth and mitotic index decreased very early with cold but no ultrastructural modification could be detected in meristematic cells after three days. For a better understanding of changes caused by low temperatures on leaf growth, it now will be essential to determine whether cell extension is affected by a chilling treatment as quickly as cell division and to clarify the effects of chilling on metabolic processes involved in cell division and cell elongation.

References

Cold stress of maize in a temperature gradient chamber

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Summary

The temperature threshold for the emergence of 10 genotypes was studied in a gradient chamber in a temperature range of 4-16 °C with a 1.3 °C gradient, and the growth of 8 genotypes was examined between 7-15 °C with a 0.8 °C gradient. The threshold for emergence was 4-7.9 °C, on average 6.2 °C. After 28 days of treatment, fresh weight showed a linear relationship between growth and temperature, the lines being characteristically distributed on the basis of the regression coefficient $b$. Line CM 174 produced hardly any growth at 7-15 °C. For the majority of the lines, it was not possible to determine the temperature characteristic of growth at 7-15 °C.

Descriptors: maize, *Zea mays* L., cold stress, gradient chamber

Introduction

The close connection between the phenophases of maize and temperature can be put to practical use now that heat unit methods have been devised. The general technique is to consider an active temperature above a threshold, generally 10°C though a lower value is also considered possible (Derieux & Bonhomme, 1982a, 1982b). The variability in the heat requirements of different genotypes (Stamp, 1984) leads to inaccuracies, which makes it difficult to adapt varieties or seed-production technology. The more exact determination of the temperature requirements of various stocks is equipment and labour-intensive, as observations have to be made in several temperature ranges. Tollenaar et al. (1979) conducted studies in the range 10-35 °C, using temperature intervals of 5 °C to compare the rate at which leaves appear.

Material and method

The gradient chamber constructed at Martonvásár is illustrated in Figure 1. In the 10 °C range, the temperature can be set with a gradient accuracy of nearly 1 °C; light can be similarly regulated by adjusting the angle of light-canopy to give a cross-gradient.

In 1981, emergence thresholds were studied for 10 genotypes at 10 temperatures in the range 4-16 °C, with a gradient 1.3 °C. The breeding material studied included 7 hybrids, 2 inbred lines and 1 open-pollinated variety. Separate pots, each sown with 5 kernels, were placed at all 10 temperature points. Observations were made from planting to emergence.

In 1984, the growth of 8 inbred lines was studied in the temperature range 7-15 °C, with a 0.8 °C gradient. The initial average height of the plants in the pots (7 plants per pot) was 3 cm.
Fig. 1. Principle of cross-gradient chamber.

when placed in the gradient chamber. Light with a uniform intensity of 30 000 lx was applied for 14 h daily (between 05:00 and 19:00) The treatment was applied for 28 days, after which the fresh weight and moisture content of the plants were determined.

Results

The threshold from planting to emergence was 4-7.9 °C (Table 1.) The time of emergence decreased from 21 to 8 days as the temperature increased from 4 to 16 °C. Acceleration of emergence was linear with temperature, and a 3-4 day period of intensive growth could be observed between 9 and 10.5 °C. Table 2 shows that the average temperature threshold was 6.2 °C, the difference between the extreme values being practically twofold. The threshold for the average European time of emergence, 13-15 days, was 9.7 °C. The data reveal that it is problematic to find a comprehensive threshold. Groups can be made, one with a lower threshold (4-6 °C) and one with a higher threshold (8 °C).

Table 1. Time of emergence (in days after sowing) at 10 temperatures (4-16 °C).

<table>
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<tr>
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Table 2. Temperature conditions for emergence.

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<th>Temperature (°C) for emergence within 13-15 days</th>
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Fig. 2. Fresh weight of young plants after 28 days of treatment.

- A 654 $r = 0.939$, $y = 1056.6 + 1.872x$
- HMv16 $r = 0.918$, $y = -790.4 + 1.579x$
- Mo17 $r = 0.952$, $y = -637.6 + 1.256x$
- HMv307 $r = 0.958$, $y = -654.4 + 1.192x$
- F 2 $r = 0.940$, $y = -620.7 + 1.152x$
- 156 $r = 0.880$, $y = -422.2 + 0.907x$
- HMv27-28 $r = 0.864$, $y = -311.6 + 0.775x$
- CM174 $r = 0.673$, $y = -126 + 0.241x$
There was a linear relationship between temperature and weight increase in the temperature range 7-15 °C for the 8 inbred lines (Figure 2). The correlation coefficient, \( r \), was 0.8-0.9, while the regression coefficient \( b \), relating growth in 28 days and temperature, ranged widely between 0.24 and 1.87 g/°C. Line Cm 174 was far different from the others in that it did not show a significant increase in weight during the 28 days at any temperature, since it was not viable below 15 °C. The moisture content of the plants indicated that the state of turgor of the other lines was adequate, whereas CM 174 gradually died (Figure 3).

The growth observed in the gradient chamber in a suboptimal temperature range (7-15 °C) revealed a fairly well definable order for the inbred lines. It was not possible to determine a characteristic temperature point either on average or for the various lines. On the basis of these preliminary studies, a certain grouping seems possible. The lines A 654 and H Mv 16 had the best tolerance to cold \( (b = 1.5-1.8 \text{ g/°C}) \); Mo 17, H Mv 307, F 2, 156 and H Mv 27-28, were among those in the medium group with \( b \) around 1.0 g/°C, and CM 174 was practically incapable of growth \( (b = 0.24 \text{ g/°C}) \).

**Conclusions**

It is not possible to find a single threshold characteristic of maize, because of variability. It is worth conducting detailed studies when economic advantages are to be expected, for instance in hybrid-seed production, and in predicting the date of maturity for the processing of sweet maize.

![Fig. 3. Moisture content of young plants after 28 days of treatment.](image)
References


Breeding for improved vegetative growth at low temperature in maize (Zea mays L.)

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Summary

Low-temperature adaptation in maize requires an adequate breeding strategy. In the Netherlands low temperature is a major growth-limiting factor in May and June. Breeders should therefore give priority to improvement of vegetative growth. Our experience in this field are presented.

The paper describes a screening method for the trait of vegetative growth per se and its use in a long-term programme for the improvement of a synthetic cold-tolerant population. Seven cycles of family selection resulted in a substantial improvement in vegetative growth. Seedlings of an advanced selection cycle showed under controlled conditions a significantly higher relative growth rate and net assimilation rate than seedlings of cv. Fronica at day/night temperatures of 15/10 °C but not at 20/15 °C. In Fronica, root growth ceased at 15/10 °C.

Breeding for improved vegetative growth at low temperature is rewarding but rather laborious and time-consuming. So there is a need for simpler breeding methods. A few options for breeders are presented.

Descriptors: maize, Zea mays L., breeding, low-temperature adaptation, vegetative growth, cold tolerance, screening methods

Introduction

Need for low-temperature adaptation Improvement of adaptation of maize to low temperature is urgent, since cultivation is rapidly extending to areas with a cooler climate. Maize is now a common crop in northern regions formerly considered unsuitable for cultivation, because of the high-temperature requirements of the crop. Cultivation in northwestern Europe extended primarily because of the suitability of the crop for forage production. The explosive increase in area could never have occurred without cold-tolerant varieties.

The central theme of this paper will be genetic improvement of cold tolerance or, more precisely, adaptation of maize to the cool maritime climate of the Netherlands.

Cold tolerance The term cold tolerance deserves further explanation. Low temperature may adversely affect maize plants from sowing until maturity. However, in agricultural practice cold conditions are usually confined to seedling growth and to early vegetative growth. Effects of low temperature may be manifold. Detailed information is presented in review papers of Miedema (1982), Crèvecoeur & Ledent (1984) and Stamp (1984). The main effects are as follows.

1. Seed and seedling rot by soil fungi in cold and wet soil. This problem has for a long time
dominated research on cold tolerance. The term 'cold tolerance' often referred to seed and seedling rot only. Large reduction in damage by rot is now possible by measures during seed processing.

2. Physiological damage before emergence. The threshold temperature of Dutch varieties is about 5 °C. Chilling treatments of 2 °C for 6 days on just germinated seedlings kills the root tips and sometimes the seedlings.

3. Reduced emergence rate. Low temperatures after sowing retard emergence.

4. Frost injury to seedlings after emergence or to mature plants at the end of the season. There is probably only little genetic variation for frost resistance so that breeding does not seem useful.

5. Physiological damage after emergence. After emergence, i.e. when seedlings are exposed to light, sensitivity to low temperature increases considerably. The most important effect is chlorosis or yellowing, which occurs at day temperatures of 10-15 °C, which are high enough for some leaf extension but too low for normal chlorophyll accumulation. Seedlings of maize are sensitive to chilling injury in bright light at temperatures between 10 and 13 °C.

6. Reduced growth rate after emergence at suboptimal temperatures. An injury threshold is hard to define as it depends on light intensity and duration of the cold conditions. A further complication is that heterotrophic (i.e. growth on seed reserves) and autotrophic growth of maize appear to have different temperature minima. The minimum for the latter is probably about 13 °C (Hardacre & Eagles, 1980). In undamaged seedlings, it has been demonstrated that leaf extension rather than net photosynthesis limits growth.

Breeding strategies Adaptation to low temperature requires an adequate breeding strategy, which should first take all relevant physiological information into account. In this respect, the impact of various effects of temperature, some injurious, on growth of maize have to be taken into consideration, including aftereffects. Breeding priorities further depend on the probability of incidence of temperature effects.

The research dealt with in this paper concerns adaptation to the weather in the Netherlands. Figure 1 summarizes data on the daily mean temperature from a weather station in the centre of the country (KNMI, 1984). It presents the proportion of days in the months April, May, June and July with daily mean temperatures below a certain temperature for a 20-year period. It thus indicates the probability of a certain effect of temperature.

In the Netherlands, maize is usually sown at the end of April. After emergence in mid-May, a 2-month period of vegetative growth follows. Cold spells inducing chlorosis are probable in May and June (Figure 1). On the other hand, temperatures are then nearly always too high for typical chilling injury, i.e. root necrosis and light-induced chilling injury.

Figure 1 also shows that during vegetative growth of maize the daily mean temperature is quite often near or slightly above the minimum temperature for autotrophic growth (about 13 °C). Genetic improvement of shoot growth at suboptimal temperatures, therefore, should be a major objective of breeding for low-temperature adaptation. Relevant selection criteria are shoot-growth rate and resistance to chlorosis.

Improvement of shoot-growth rate

Introduction Selection for improved shoot growth at suboptimal temperatures is hampered by several problems. The most relevant is undoubtedly the high coefficient of variation for shoot dry weight usually found in plants grown in the field and even in controlled conditions.
Genetic variation in heterotrophic shoot growth is a second complicating factor, since heterotrophic and autotrophic shoot growth are interwoven. Consequently differences in autotrophic shoot-growth rate may be somewhat masked before seed depletion.

Most literature on improvement of shoot growth at low temperature deals with early seedling growth (e.g. Eagles, 1982; Eagles & Hardacre, 1979; Menkir & Larter, 1985; Mock & Bakri, 1976; Mock & Eberhart, 1972; Mock & McNeill, 1979; Mock & Skrdla, 1978). Improvement of vegetative growth at low temperature has received little attention in breeding research. At SVP, however, we have concentrated most of our effort on improving this trait.

**Screening method** Genetic differences in ability to grow at low temperature are nearly always visible in the Netherlands, if maize is sown on time. Our breeding approach has consequently been to screen for improved vegetative growth in simple replicated sowing date trials (Dolstra & Jongmans, 1981). Each test consists of six one-row microplots of 10 plants, equally distributed over an early and a normal sowing date in the first and last ten days of April, respectively. The trial is laid out according to an appropriate statistical design, which facilitates corrections for block effects. In the field tests, data are collected for each microplot on proportion of plants emerging, emergence date and shoot biomass. With an interval of 1-2 weeks, the latter trait is rated twice visually when 6-8 leaves (stage 4 on scale of Hanway (1970)) are visible on a 1-9 scale: 1, poor vigour, 9 excellent vigour. Subsequent selection before silking is primarily on the basis of 4 mean ratings of vigour for each entry, i.e. 2 mean ratings for each sowing date. Within each set of data, the entries are ranked from poor to excellent growth. An index is finally calculated for each entry by summation of the vigour rankings by the method of Mock & Skrdla (1978), in order to facilitate selection.

Genetic differences in growth rate among maize introductions and families are readily de-
ected in spring by the screening method. Critical in the procedure is the time of the vigour rating, since time is needed before differences in autotrophic growth become visible. Vigour ratings are an acceptable alternative for determinations of shoot dry weight, because both measures are highly correlated. In a 1979 field trial, for instance, we found a correlation coefficient of 0.94 \( (P < 0.01) \) between individual plant ratings and shoot dry weight.

In a small-scale selection trial, the test has resulted in a gain in vegetative growth detectable over a wide range of rather cool environments (Dolstra & Jongmans, to be published). So selection resulted in a general improvement in shoot growth under suboptimal conditions. There are no serious objections to testing in only one environment, since no significant genotype × environment interactions were found.

**Population improvement**  A recurrent selection programme to improve vegetative growth is in progress in the synthetic cold-tolerant source population, SVP-PD, with the screening method. Since 1978, we have completed one cycle of family selection per year in this population. Initially the selection method was not fully fixed, but since 1982 we use only full-sib family selection. More information on the selection procedure was presented at the meeting of the Maize and Sorghum Section of Eucarpia in Montreux (Dolstra & Jongmans, 1981). Throughout the whole programme, we maintained a large effective population \( (N_{eq} >> 50) \) by testing over 200 families per cycle and using a mild intensity of selection (females 15-20%; males variable).

Figure 2 indicates the response to selection for vegetative growth. The frequency distributions of the selection cycles \( C_0, C_4 \) and \( C_6 \) are based on standardized family means for vegetative growth, using the data of the second rating from the early sowing date of 1978, 1982 and

![Figure 2](image-url)
1984, respectively. The data were equated between years by subtracting the mean ratings of the control varieties LG11 and Fronica from the family means and dividing the difference by the standard error of family means. Standardization allows comparisons between selection cycles.

Figure 2 shows a gradual shift of the population mean to the right for subsequent selection cycles, indicating a steady improvement in vigour. The shape of the curves, on the other hand, was not much affected by selection. So the prospects for further improvement are still favourable. The striking difference between the control varieties and advanced selection cycles implies a large difference in shoot dry weight. For instance, in 1984, an exceptional cold year, the shoot dry weight of Cycle 6 was over 50% more than that of cv. Fronica when the plants had 5-7 visible leaves. Since the performance of cv. Fronica and the source population C0 were similar, selection must have resulted in a substantial improvement in vegetative growth under cool conditions.

Causes of improved vegetative growth Two studies under controlled conditions gave more information on the causes of improved vegetative growth of population SVP-PD. Cycle 6 and cv. Fronica were used in growth analysis at day/night temperatures of 15/10 °C and 20/15 °C with a photoperiod of 16 h and a photosynthetically active irradiance of about 60 W m². Shoot dry weight and leaf area were periodically determined; for each temperature treatment, about 36 plants were used for each sampling date.

Figure 3 shows that shoot dry matter accumulated much faster at 20/15 °C than at 15/10 °C. At 20/15 °C, the growth curves of the two groups of plants were similar. At 15/10 °C, however,
shoot dry matter accumulated faster in SVP-PD than in cv. Fronica; at the end of the growth period, shoot dry weight of the two groups was significantly different at $P < 0.01$. The data show that selection for improved vegetative growth improved shoot growth at 15/10 °C but not at 20/15 °C.

The leaf area ratio, i.e. leaf area divided by mass of shoot dry matter, was somewhat lower in SVP-PD/C6 than in cv. Fronica with both temperature treatments. This implies that the net assimilation rate of both groups of plants, i.e. increase in shoot dry weight divided by leaf area, differed at low temperature even somewhat more than relative growth rate, since relative growth rate = net assimilation rate $\times$ leaf area ratio. The lower leaf area ratio of SVP-PD/C6 was associated with thicker leaves and larger leaf sheaths.

In a second experiment, we studied the development of the shoot-to-root ratio for SVP-PD/C7 and cv. Fronica in gravel culture. The growing conditions were the same as in the previous trial. Shoot and root dry weight were subsequently determined in the stage of the 2nd-, 3rd- and 4th-leaf (Figure 4). With development, the shoot-to-root ratio rose. The distribution of dry matter over shoot and root was in SVP-PD/C7 only little and in cv. Fronica seriously affected by a decrease of temperature. The reason for lack of root growth at low temperature in cv. Fronica is unknown. The finding, however, agrees quite well with observations of Stamp (1984), who found that root development of cold-susceptible genotypes was much more hampered at low temperature than shoot development.

Leaf or cell elongation may be a major factor in differences in shoot growth at low temperature (Miedema, 1982; Stamp, 1984). Elongation of the 3rd leaf was at 20/15 °C 3% and at 15/10 °C 7% better in SVP-PD/C7 than in cv. Fronica. The difference in shoot-growth rate between the two groups of plants was to some extent reflected in the leaf-elongation rate. So this trait might be useful for screening of growth at low temperature. The measurements are simple and non-destructive.

**The impact of improved vegetative growth** Selection for improved vegetative growth will result in an increase of biomass production by shortening the period from emergence to closing of the canopy. The question arises whether this gain is sufficient to justify intensive breed-

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**Fig. 4.** Course of mass of shoot dry matter of seedlings of cv. Fronica (*) and the synthetic cold-tolerant population SVP-PD Cycle 7 (●) grown in gravel culture under controlled conditions at 20/15 or 15/10 °C (day/night). For each temperature treatment, seedlings were weighed at three dates (2nd–4th leaf stage).
Fig. 5. Shoot dry-matter accumulation in the field in two flint populations (solid lines) and two dent populations (dotted lined). Trial 1984 in East Flevoland sown 3 May. The final plant density was 9/m².

Figure 5 indicates what might be achieved by intensive selection. It summarizes the data of a crop-growth study with four breeding populations, i.e. advanced cycles of two selection programmes for improved vegetative growth (SVP-PD and SVP-PE, synthetic flint and dent populations, respectively) and two breeder’s populations. The experiment covers the period from 3 May to 26 July, in which plant dry weights were periodically measured. The daily mean temperature was in that period about 1.2 °C below normal. The cool weather prolonged vegetative growth considerably, so the linear crop-growth phase was not reached even by the end of the experiment. The differences in initial growth finally resulted in striking differences in shoot dry weight at the end of the growth analysis. In yield of dry matter, the best and the poorest population, SVP-PD/C6 and VDH Dent, differed by 700 kg/ha (0.7 kg/m²). The cold-tolerant populations were better than their unselected flint or dent counterparts. Figure 5 indicates that breeders should not make their vigour ratings too early in the year.

The final dry-matter yield of the four populations under study was determined in the same trial 160 days after sowing (Table 1). The differences in dry weight per plant between the populations were similar of more pronounced than those observed 84 days after sowing. The ranking of the populations for mass of shoot dry matter per plant, however, did not change in the following growth phases. The improved populations had also produced a larger proportion of the final yield in the first 84 days of the experiment than the control populations. So selection for vegetative growth under cool conditions increased dry-matter yield and improved yield stability.
Table 1. Comparison of the dry weight of total aerial parts (shoot) of four breeding populations at 84 days and of ear and stover at 160 days after sowing. For more detailed information see Figure 5.

<table>
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<th>b. Final dry weight per plant (g) ear</th>
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<th>a/b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flint</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SVP-PD/C6</td>
<td>14.3</td>
<td>44.0</td>
<td>81.7</td>
<td>0.114</td>
</tr>
<tr>
<td>VDH Flint</td>
<td>9.5</td>
<td>46.7</td>
<td>76.9</td>
<td>0.077</td>
</tr>
<tr>
<td>Dent</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SVP-PE/C2</td>
<td>7.9</td>
<td>34.8</td>
<td>75.5</td>
<td>0.067</td>
</tr>
<tr>
<td>VDH Dent</td>
<td>6.6</td>
<td>38.2</td>
<td>66.9</td>
<td>0.063</td>
</tr>
</tbody>
</table>

Improvement of resistance to damage

Introduction  The differences in ability to grow in cool conditions may be attributed to two main factors: leaf extension and resistance to chlorosis. The relative contribution of those factors to accumulation of dry matter is unknown. If resistance to chlorosis is a major factor, breeding for it may be a useful approach, since screening is much simpler than for field performance. Besides resistance to chlorosis, resistance to chilling-induced root necrosis may be interesting. Screening for this type of chilling resistance is simple but as yet there is little evidence for its relevance to biomass production (Miedema et al., in press). Resistance breeding to these cold-tolerance traits is under study in advanced selection cycles of the two cold-tolerant populations. We are selecting in a positive and a negative direction. The aim of this selection trial is to establish the relevance of the traits under selection. Data are tentative.

Resistance to chlorosis  Maize is screened for resistance to chlorosis in growth cabinets. Plants are grown until emergence in small jiffy pots at 20 °C. The environmental conditions are subsequently changed. The air temperature is lowered to 15/10 °C (day/night). Photosynthetically active irradiance is about 60 W m⁻² during the light phase of 16 h. Selection is possible by the 2nd or 3th leaf.

In the cold-tolerant synthetic flint population, SVP-PD/C6, we have completed two cycles of mass selection by this procedure. In Cycle 2, the temperature was lowered to 14/10 °C (day/night). Vigorous plants were selected with either green of yellow colour. Our impression is that this population responded quite well to selection. The source population was, however, already rather resistant, since it is in part based on some Latin American and Portuguese accessions with excellent resistance to chlorosis at low temperature. Since the genetic variation for this trait is much less in the other population under selection, i.e. the dent population SVP-PE/C3, selection seems less promising.

In conclusion, mass selection according to the outlined procedure is a simple, quick and probably efficient way of improving breeding populations.

Resistance to chilling induced root necrosis  A prolonged period with temperatures of 0-5 °C induces necrotic root lesions on main and auxiliary roots of seedlings (Miedema, 1982;
Miedema et al., in press). Root necrosis occurs in the elongation zone and probably adversely affects development and function of the root system.

Roots are screened for resistance to necrosis as follows. Germination of seeds occurs in large plastic trays with moist perlite for 3 days at 20 °C. The seedlings are subsequently kept for 6 days at 2 °C and 3 days at 20 °C to induce root necrosis. The damage to the main roots of individual seedlings is readily rated on a scale 1-9 (1, necrotic roots; 9 healthy roots).

The two populations under selection had wide genetic variation in susceptibility to root necrosis. Preliminary research had shown that this trait was highly heritable and amendable by mass selection. We have now completed one cycle of mass selection in both populations. Most plants recovered from the stress treatment, even the sensitive ones.

Impact of resistance breeding Breeding conditions are favourable for a rapid improvement of resistance to chlorosis and root necrosis. There is sufficient genetic variation in both traits and screening techniques are simple, rapid and non-destructive. So breeders could use the winter season to select for resistance without delay in other selection activities. Mostly chlorosis and root necrosis do not kill seedlings or young plants. The impact of resistance on plant growth is consequently the main reason for resistance breeding. Since resistance to chlorosis increases growth, breeding for resistance to is certainly justified. The impact of resistance to root necrosis, on the other hand, is still unknown.

Conclusions

Breeding for improved vegetative growth at low temperature is rather laborious and time consuming because of the high environmental error and the polygenic inheritance of shoot dry weight. Nevertheless, significant improvements in shoot growth rate can be generated by recurrent selection under cool conditions. Improved vegetative growth increases final shoot dry weight per plant and improves yield stability. So breeding efforts are certainly rewarding despite these difficulties.

Striking characteristics of a population with improved vegetative growth were a low leaf-area ratio, attributable to thicker leaves and long leaf sheaths and more rapid increase of leaf area and length. So leaf elongation seems a suitable non-destructive criterion of selection. A well balanced shoot-to-root ratio is probably necessary for maintenance of shoot growth at low temperature. The stopping of root growth at low temperature, observed in young seedlings of the commercial hybrid cv. Fronica deserves further investigation.

In selection under cool conditions, no distinction was made in the field between low-temperature damage, such as chlorosis, and growth rate above the injury threshold. Breeding for resistance to chlorosis and possibly chilling-induced root necrosis, may be an additional or even an alternative approach for direct improvement of shoot growth.

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Simple method of evaluating cold resistance in maize lines early in development

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Summary

Based upon the analysis of the capacity of seeds of maize lines to germinate at low temperatures (potential cold resistance) and to preserve germinating power after the cessation of germination (maintenance of germinating power), an index of cold resistance was constructed. According to these indices, six classes have been formed for cold resistance.

Descriptors: maize, Zea mays L., low temperature, germination, cold resistance

Introduction

Maize is a thermophilic crop. A low environmental temperature lessens its yield and restricts its area of cultivation. The principal way of overcoming these impediments is to create early maturing cold-resistant varieties and hybrids. For solution of this problem, one must develop a simple and convenient method of evaluating cold resistance in maize lines, a method that can be used for the selection of suitable initial material.

Taking into consideration the vast diversity of maize lines and the labouriousness of direct methods of evaluating cold resistance in the field, many research workers are turning to development and use of a laboratory cold test.

One of the best known, most widely used and simplest methods of evaluating cold resistance early in development is germination in a cold environment or the cold test (e.g. Woodstock, 1976; Isely, 1950; Crosier, 1957; Hoppe, 1955; Rice, 1960; Koch, 1970; Protosenko & Mishustina, 1962; Stepanov & Shatilov, 1959; Kalyn, 1970). This test has many variants.

The purpose of our work was to select the most informative method of evaluating cold resistance in the samples from the All-Union Institute’s collection.

Methods

Seeds were germinated at 7 °C in large sterilized Petri dishes on three layers of humid filter paper for 28 and 36 days and in rolls of filter paper at 10 °C for 10 and 20 days. The seedlings were allowed to grow for 2-4 days at 25 °C. The tests had four replicates. The control germination was at 25 °C in rolls of filter paper (Likhachev & Zhukova, 1978).

Fields trials were conducted by the methods of the Institute. The maize was sown in 1983 and 1984 at two dates: an early date and an optimum date.
Results

The temperature of 10 °C was more suitable for germination under cold conditions than 7 °C. At the lower temperature, there were no normally formed healthy seedlings, even after 36 days of germination. So the temperature of 7 °C is below the biological minimum for germination of maize. We conducted all further investigations at 10 °C.

According to Koch (1970), the extent of damage by low temperature depends not only on the temperature as such but also on its duration. Our tests showed that germination for 20 days at 10 °C was the best way of distinguishing maize samples for germination under suboptimal conditions. The differences in behaviour of seeds and seedlings after a period of cold caused us to evaluate the germination power three days after the end of the cold treatment too. Table 1 presents the scheme of our modification of the method of germinating seeds in a cold environment. For the control samples, number of germinated seeds was assessed on the fifth day ($N_0$). The germination of the treated samples was assessed on the 20th day ($N_t$) and after three days with optimum temperature a final evaluation of seed germination was carried out ($N_2$).

The first estimate, which we called potential cold resistance, was calculated with the formula $N_t/N_0$ and the second for maintenance of germination power with the formula $N_2/N_0$.

Since seeds of maize lines can germinate at low temperatures and can maintain germination power after cessation of germination, we designated cold resistance by an index whose first digit expresses the potential cold resistance and whose second digit expresses the maintenance of germination power.

Taking into account these two values and the significance of each value, we divided all the lines studied into the following six groups (Table 2):

- Group I, samples with a high potential cold resistance ($\geq 66\%$) and good maintenance of germination power ($\geq 70\%$): index 1.1
- Group II, samples with an average potential cold resistance and good maintenance of germination power: index 2.1
- Group III, samples with a high potential cold resistance but average maintenance of germination power ($\geq 50\%$): index 1.2
- Group IV, samples with a low potential cold resistance (less than 33%) but good maintenance of germination power: index 3.1
- Group V, samples with indices 2.2, 3.2 and 1.3
- Group VI, samples with average or low potential cold resistance and low maintenance of germination power: indices 2.3 and 3.3.

When allotting the lines to the groups, we proceeded from the greater significance of maintenance of germination power, that is the capacity of seeds to remain viable after a long spell of low temperature.

The grouping of lines based on field data for germination and on the evaluation of seedlings

Table 1. Scheme of the method proposed for cold germination of maize. Substrate: paper rolls with correx, 0.16 m x 1.2 m

<table>
<thead>
<tr>
<th>Variants</th>
<th>Germination at 10 °C</th>
<th>Germination at 25 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>5 days</td>
</tr>
<tr>
<td>Treated</td>
<td>20 days</td>
<td>3 days</td>
</tr>
</tbody>
</table>
### Table 2. Allocation of maize lines to the groups of cold resistance.

<table>
<thead>
<tr>
<th>Line</th>
<th>Potential cold resistance (%)</th>
<th>Maintenance of germination power (%)</th>
<th>Index of cold resistance</th>
<th>Group for cold resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>H147</td>
<td>77</td>
<td>93</td>
<td>1.1</td>
<td>I</td>
</tr>
<tr>
<td>W85</td>
<td>70</td>
<td>71</td>
<td>1.1</td>
<td>I</td>
</tr>
<tr>
<td>Ny-2R</td>
<td>79</td>
<td>90</td>
<td>1.1</td>
<td>I</td>
</tr>
<tr>
<td>VIR-44,M</td>
<td>35</td>
<td>89</td>
<td>2.1</td>
<td>II</td>
</tr>
<tr>
<td>502</td>
<td>34</td>
<td>89</td>
<td>2.1</td>
<td>II</td>
</tr>
<tr>
<td>092</td>
<td>48</td>
<td>75</td>
<td>2.1</td>
<td>II</td>
</tr>
<tr>
<td>FS23/666/74</td>
<td>70</td>
<td>57</td>
<td>1.2</td>
<td>III</td>
</tr>
<tr>
<td>D-Be-15</td>
<td>91</td>
<td>61</td>
<td>1.2</td>
<td>III</td>
</tr>
<tr>
<td>D-Be-19</td>
<td>93</td>
<td>69</td>
<td>1.2</td>
<td>III</td>
</tr>
<tr>
<td>L</td>
<td>4</td>
<td>77</td>
<td>3.1</td>
<td>IV</td>
</tr>
<tr>
<td>Mo-17</td>
<td>1</td>
<td>77</td>
<td>3.1</td>
<td>IV</td>
</tr>
<tr>
<td>346</td>
<td>22</td>
<td>82</td>
<td>3.1</td>
<td>IV</td>
</tr>
<tr>
<td>AD-36</td>
<td>43</td>
<td>63</td>
<td>2.2</td>
<td>V</td>
</tr>
<tr>
<td>W64A</td>
<td>10</td>
<td>59</td>
<td>3.2</td>
<td>V</td>
</tr>
<tr>
<td>D-Be-36</td>
<td>82</td>
<td>25</td>
<td>1.3</td>
<td>V</td>
</tr>
<tr>
<td>D-Be-11</td>
<td>43</td>
<td>4</td>
<td>2.3</td>
<td>VI</td>
</tr>
<tr>
<td>A96</td>
<td>27</td>
<td>38</td>
<td>3.3</td>
<td>VI</td>
</tr>
</tbody>
</table>

in the 5-6 leaf stage (sown in three ecological zones) largely coincided with the grouping of lines according to the laboratory data. The evaluation of real cold resistance in the field and its correlation with laboratory predictions confirms the expediency of using rapid laboratory tests for the study of this trait in the collection of self-pollinated lines.

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Improvement of feeding value
Improving the nutritive value of forage maize

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Summary

Consequences for digestion and utilization of feeds by ruminants are described on the basis of the molecular, anatomical, histological and morphological structure, and the physiological behaviour of whole maize plants and crops. The prospects of improving the nutritional quality by breeding and management are also discussed. On the molecular, histological and morphological levels, there is a wide variation in digestibility. However the variations at the whole-plant level and at the whole-crop level are smaller and are largely due to genetic variation. Physiological factors limit the practicality of breeding for higher content of protein and minerals. Genetic variations in digestibility and in intake of dry matter are wide and these characteristics can be improved by breeding. The best way of improving the whole-plant digestibility by breeding seems to be to select for a high cellwall digestibility of the vegetative parts. Low digestibilities of forage maize in some countries are to some degree attributable to environmental conditions but are more attributable to choice of hybrid.

Descriptors: forage maize, Zea mays L., protein content, digestibility, breeding, feed intake, cellwall, growth, heritability

Introduction

Forage maize is grown for ruminants to produce meat and milk. The value of the crop is partly determined by the ratio of animal production to mass of maize silage, i.e. the efficiency of the conversion of forage to animal product. This conversion is affected by the digestibility of the forage, the animal's intake and the efficiency of feed utilization. These factors are interrelated and all are influenced by both animal and plant characteristics. Intake and feed efficiency can be measured only in very expensive animal trials. Digestibility can be predicted accurately by incubation in vitro of ground forage in rumen fluid (Deinum et al., 1984).

Recent research at our department has shown that digestibility is a heritable trait that can be improved by breeding. Considerable genetic variation is available. However this variation has not yet been explored. Lack of attention to digestibility has resulted in new hybrids with lower digestibility in the Netherlands. This paper will discuss some aspects of the nutritive value of forage maize and the prospects of improving digestibility.

Breeding objectives for improving nutritive value

Table 1 presents the required feeding value for some classes of production and the average feeding value of maize silage in the Netherlands. Forage maize is primarily used as an energy source. It is insufficient to meet the protein requirements of highly productive livestock and is
Table 1. Minimum requirements of different classes of livestock for content of crude protein in dry matter and apparent digestibility of the diet, and average quality of ensiled maize in the Netherlands.

<table>
<thead>
<tr>
<th>Livestock type</th>
<th>Production (kg/d)</th>
<th>Intake of dry matter (kg/d)</th>
<th>Content of crude protein (%)</th>
<th>Digestibility of organic matter (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cow (wt 500)</td>
<td></td>
<td></td>
<td>6.3</td>
<td>7.2</td>
</tr>
<tr>
<td>cow (wt 500, milk)</td>
<td>10</td>
<td>10</td>
<td>13.7</td>
<td>65</td>
</tr>
<tr>
<td>bull (wt 400, carcass)</td>
<td>25</td>
<td>10</td>
<td>16.3</td>
<td>83</td>
</tr>
<tr>
<td>Average, maize silage</td>
<td></td>
<td>10</td>
<td>9.4</td>
<td>65</td>
</tr>
</tbody>
</table>

deficient in some minerals and vitamins. Nevertheless, Struik (1983a) stated that improvement of the composition of the non-structural material in the plant should be of low priority as a breeding objective. For physiological reasons, it is also undesirable to alter the amounts of certain cell solubles such as nitrogen and minerals.

Nitrogen and minerals are taken up from the soil and are dispersed over the dry matter produced. The absorbed nitrogen is assimilated into protein. Thus, protein content, as a mass fraction, is the yield of protein divided by the yield of dry matter. This mass fraction can therefore be increased by an increased yield of protein or a decreased yield of dry matter. Since the latter is not acceptable, uptake of nitrogen should be increased or even doubled. This greater uptake of nitrogen is only possible if the supply of nitrogen in the soil is adequate and if the root system is excellent at absorbing nitrogen. The same holds for the minerals. Improvement of protein and mineral content is therefore very difficult. Breeding for higher yield of dry matter may even lead to lower contents of protein and minerals.

In the diet, the low contents of protein and minerals in forage maize must be made good by supplementing with forages and concentrates rich in protein and minerals. This can easily be done in intensive feeding systems and gives scope for the digestibility of forage maize to be improved and for intake by the animal to be increased. Digestibility, dry-matter content and intake should be the main objectives of improving quality of forage maize. In this paper, we will ignore dry-matter content.

Improving intake and digestibility

Crop science and animal science are different disciplines. Animals are fed with chopped maize particles but digest the molecules and tissues of this feed. Crops of maize are grown in different climates and countries under different management systems and at different cultural practices. Crops are communities of plants, usually of one species, with a specific genetic and phenotypic background. Plants are composed of different morphological fractions. These fractions are composed of different tissues, which in turn are composed of cells. Cells consist of walls and contents, both composed of different kinds of molecules. These molecules may be digested and used by the animals.

In this paper, we shall follow this route in reverse, starting from the digestion of molecules, through cells, tissues, plants to crops and regions, and we shall end with prospects of improving quality.
Molecules

The molecules found in maize and other forages, can be characterized in different groups (Table 2). Some classes of molecules, such as sugars and organic acids, are completely digestible in ruminants. Proteins are very digestible; lignin, cutin, silica and tannins are almost indigestible. The digestibility of cellulose and hemicellulose varies, depending partly on whether they are encrusted with lignin. Forage-maize digestibility can be improved considerably if this variation is used in breeding programmes.

Crude fibre and nitrogen-free extract, estimated in the proximate analysis, are mixtures of classes of molecules.

Cells

Plants are built up from differentiated cells, each with its own structure and function. These cells are composed of the various classes of molecules. Figure 1 presents the average composition of forage maize plants in the Netherlands according to the old proximate analysis (Weende analysis) and to the more modern fractionation by van Soest’s methods (1967). The molecules of the cell contents account for more than 50% of the whole dry mass of the crop. Cell contents are almost completely digestible in the ruminant, whereas the cell walls are only partly fermented and digested. The potential digestibility of cell walls is partly correlated with their lignin; rate of cell-wall digestion depends mainly on other partly unknown physico-chemical factors.

Figure 1 shows that crude fibre is a mixture of cellulose and lignin, and that nitrogen-free extract is a conglomerate of completely digestible organic acids and sugars, indigestible lignin and partly digestible hemicellulose. Consequently, crude fibre and nitrogen-free extract are of little use for an accurate estimate of the digestibility of forage. Lignin is a poorly defined class of polyphenols. It is interwoven with the cellulose and hemicellulose molecules and protects them from digestion: when lignin content is low, digestion is almost complete; when content is high, digestion is incomplete. Monomeric phenols possibly have little effect on digestion, whereas polyphenols greatly inhibit rate and extent of digestion of organic matter (see the contribution of Engels & Cone, these proceedings).

<table>
<thead>
<tr>
<th>Molecules</th>
<th>Cell part</th>
<th>Digestibility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minerals</td>
<td>lumen/cell wall</td>
<td>10-95</td>
</tr>
<tr>
<td>Protein</td>
<td>lumen/cell wall</td>
<td>90-99</td>
</tr>
<tr>
<td>Organic acids</td>
<td>lumen</td>
<td>100</td>
</tr>
<tr>
<td>Sugars and starch</td>
<td>lumen</td>
<td>100</td>
</tr>
<tr>
<td>Lipids</td>
<td>lumen</td>
<td>90-100</td>
</tr>
<tr>
<td>Lignin</td>
<td>cell wall</td>
<td>0</td>
</tr>
<tr>
<td>Cellulose</td>
<td>cell wall</td>
<td>20-100</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>cell wall</td>
<td>20-100</td>
</tr>
<tr>
<td>Cutin, tannins</td>
<td>cell wall/lumen</td>
<td>0</td>
</tr>
<tr>
<td>Silica</td>
<td>cell wall</td>
<td>0</td>
</tr>
<tr>
<td>Crude fibre</td>
<td></td>
<td>20-95</td>
</tr>
<tr>
<td>Nitrogen-free extract</td>
<td></td>
<td>30-90</td>
</tr>
</tbody>
</table>
The preceding information on digestion can be written as:

\[ \% D_{om} = \% \text{cell contents} \times 0.98 + \% cwc \times D_{cwc} - \text{endogenous excretion} \]

In this:

\( \% D_{om} \) = apparent digestibility of organic matter,

\( \% \text{cell contents} \) and \( \% \text{cell-wall constituents} \) are percentages of organic matter,

0.98 = true digestibility of cell contents,

\( D_{cwc} \) = digestibility (true) of cell-wall constituents,

endogenous excretion is non-feed organic matter added to the feed by the animal and excreted in the faeces together with undigested feed (% of feed intake).

Dutch silage maize shows, on average, the following characterization of the formula:

\[ 73.5 = 54 \times 0.98 + 46 \times 0.64 - 9 \]

(The digestion in ruminants can be imitated in vitro by incubating the ground forage with rumen microflora (e.g. Deinum et al., 1984).)

To improve digestibility of plant material, most emphasis should be put on the mass fraction of cell walls and their digestibility. No chemical analysis can fractionate the molecules into digestible and indigestible fractions. Rumen microflora must be used to do this.

**Tissues**

Cells are grouped into tissues throughout the plant. Parenchyma often consists of large thin-walled cells (as in pith or leaf mesophyll). Its cell wall is low in lignin and easily and rapidly digested when young, but the cell-wall digestibility is lower in old plants. In sclerenchyma, cells are often long, with a small lumen and a thick lignified wall. These cell walls are digested slowly and to a limited extent (see the contribution of Engels & Cone, these proceedings). In vascular bundles, phloem is usually thin-walled and well digestible, but xylem vessels are highly lignified and poorly digestible. The cuticle of the epiderm is also poorly digestible.

The kernel (caryopsis) is made up of a seed coat (pericarp), embryo and endosperm. Each of these parts has a different hereditary make-up. The endosperm comprises the largest part of
the kernel and consists mainly of starch in 'normal' genotypes. The outermost layer of the endosperm is the aleurone layer. Cells in these tissues have a large and filled lumen and their walls are fairly digestible, except for the pericarp, which can resist rumination and digestion very well.

To sum up, there is a wide range in true digestibility between tissues, from hardly digestible (about 10%) to almost completely digestible (about 99%). Breeding and management may influence the digestibility of these tissues.

**Morphological fractions**

Plant tissues are grouped in varying proportions into different morphological fractions such as leaves, stems and kernels. Leaves, pith and kernels are rich in parenchyma. Stems and mid-ribs are rich in sclerenchyma and vascular bundles. Table 3 shows that some fractions, such as the kernels, parts of the leaves and the pith of the stem are highly digestible, whereas the mid-rib of the leaf, the sheath and the rind of the stem are poorly digestible, presumably because of the large proportion of sclerenchyma and vascular bundles. Even within the leaf fraction, there is a wide variation. The first small leaves have a low cell-wall content and a high digestibility of cell wall and of organic matter. The large and small leaves that develop higher up the stem have a much higher cell-wall content and a much lower digestibility of cell wall, presumably because of the large mass fraction of mid-rib and the larger proportion of vascular bundles (Deinum, 1976). Although there is still a wide range in digestibility among the different morphological fractions, the range is much smaller (about 40-85%) than between the various tissues, and breeding and management may influence the proportion and digestibility of these fractions.

**Plants**

Plants are composed of different morphological fractions. Consequently, the proportion of the various morphological fractions and their composition determine the chemical composition and digestibility of the whole plant. Morphological composition changes dramatically during growth, because of the succession of development of leaves, stem, ear and kernel (Figure 2), and so does the production of the various tissues. Consequently, digestibilities of

<table>
<thead>
<tr>
<th>Part</th>
<th>Contribution to plant yield (%)</th>
<th>Dry-matter content (%)</th>
<th>Digestibility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tassel</td>
<td>1.3</td>
<td>53.8</td>
<td>47.6</td>
</tr>
<tr>
<td>Kernel</td>
<td>43.5</td>
<td>43.1</td>
<td>88.6</td>
</tr>
<tr>
<td>Cob</td>
<td>10.1</td>
<td>33.1</td>
<td>68.4</td>
</tr>
<tr>
<td>Husk + shank</td>
<td>11.8</td>
<td>24.8</td>
<td>65.5</td>
</tr>
<tr>
<td>Mid-rib</td>
<td>2.1</td>
<td>26.8</td>
<td>56.3</td>
</tr>
<tr>
<td>Leaf mesophyll</td>
<td>9.2</td>
<td>35.9</td>
<td>76.7</td>
</tr>
<tr>
<td>Leaf sheath</td>
<td>4.5</td>
<td>28.8</td>
<td>55.6</td>
</tr>
<tr>
<td>Rind of stem</td>
<td>13.2</td>
<td>18.3</td>
<td>54.4</td>
</tr>
<tr>
<td>Pith of stem</td>
<td>4.3</td>
<td>12.2</td>
<td>73.6</td>
</tr>
<tr>
<td>Whole plant</td>
<td>100</td>
<td>29.3</td>
<td>75.4</td>
</tr>
</tbody>
</table>
cell wall and of organic matter also decline until the plant matures and has produced all cell wall material (shortly after grain set). Thereafter, digestibility of cell wall declines gradually, possibly because of further encrustation with lignin and because of changes in the physical structure of the cell wall. However the digestibility of organic matter may remain constant or may even increase, according to whether completely digestible non-structural carbohydrate continues to be produced. If such production is scanty because of low irradiance, premature leaf senescence or water shortage, this production cannot counterbalance the decline in cell-wall digestibility (Struik, 1982; 1983a,b; Struik & Deinum, 1982).

At high plant density, a greater proportion of dry matter is laid down as cell wall in the
stover and so digestibility of organic matter is less (Struik & Deinum, 1982). If pollination is poor or absent, kernels do not develop properly and morphological composition is altered, because the carbohydrate that normally accumulates in the grain is then retained in the stover. Therefore, differences in grain set and grain filling have only limited effect on dry-matter production (cell contents and cell wall) and on digestibility (Figure 3). However, the range in digestibility caused by differences in the success of pollination is comparable with the range that can be obtained by variation in cultural practice, the hybrid chosen or climatic conditions. In contrast, removing the ear completely at silking causes a dramatic reduction in yield and quality.

Since the morphological composition of maize plants varies little at the ensiling stage, digestibility varies little (65-80%) between plants. Nevertheless, these variations are significant and are affected by environmental and genetic factors. Within a uniform crop, the plant-to-plant variation is probably much less (only a few per cent), and is not much more than the error of estimation for digestibility in vitro.

Environmental factors

Extensive research at our department (Deinum & Knoppers, 1979; Struik, 1983a) has shown that several factors affect digestibility of organic matter (Table 4). Variation in digestibility of organic matter of the maize plant is related to the production and digestibility of the cell wall in the stover and in the ear and to the amount of cell contents in the whole plant. Consequently, we found good correlations between the content of cell wall and the digestibility of organic matter.

![Graph showing the effect of success of grain set on mass and digestibility (D_{om}) of a silage maize plant (Struik, 1983b).](image)
Table 4. Effects of several external factors on digestibility of the maize plant (after Struik, 1983a).

<table>
<thead>
<tr>
<th>Factor</th>
<th>Effect on</th>
<th>%D_{om}</th>
<th>% cwc</th>
<th>%D_{cwc}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>high irradiance</td>
<td>+</td>
<td>-</td>
<td>±</td>
<td></td>
</tr>
<tr>
<td>high temperature</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>abundant water</td>
<td>± or +</td>
<td>± or -</td>
<td>±</td>
<td></td>
</tr>
<tr>
<td>long day</td>
<td>-</td>
<td>+</td>
<td>±</td>
<td></td>
</tr>
<tr>
<td>Crop</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>increased plant density</td>
<td>-</td>
<td>+</td>
<td>±</td>
<td></td>
</tr>
<tr>
<td>late sowing</td>
<td>-</td>
<td>+</td>
<td>±</td>
<td></td>
</tr>
<tr>
<td>delay of harvest</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

---, -, ±, +: highly negative, negative, hardly any, positive.

Genetic variation

Such good correlations between content of cell wall and the digestibility of organic matter have not been found in varietal trials. The high digestibility of the mutants with brown mid-ribs, produced at Purdue University (Muller et al., 1972) was the first indication of the great genetic variation in digestibility of maize plants. Later Gallais et al. (1980) confirmed this higher digestibility of such mutants in French hybrids and also found a greater intake and feed efficiency in ruminants.

Noting these findings, Deinum & Bakker (1981) started investigating the digestibility of hybrids planted in varietal trials and found substantial differences in digestibility of organic matter between hybrids (e.g. 72.4-76.6% in 1978). They also discovered that %D_{om} of the whole plant was only partly correlated with ear content (Figure 4), presumably because of genetic differences in translocation of cell contents from the stover to the ear (note the positive correlation between content of cell wall in stover and of ear in whole plant) and of the small range in ear content. Path coefficient was somewhat better. However, there was a great positive correlation between %D_{om} of whole crop and %D_{cwc} of stover. Path coefficient was somewhat smaller. Similar results were found in other years. Interactions hybrid x site and hybrid x year were small. These genetic differences were only found for digestibility in vitro, not for content of crude fibre or cell wall. This indicates that genetic variation in cell-wall digestibility was greater in the group of hybrids tested in the Netherlands than was content of cell wall.

Similar results were found when studying the digestibility of a limited number of hybrids including three versions with brown mid-ribs of well known French varieties (Table 5). Eta Ipho, notoriously low in digestibility of organic matter in all trials, also had the lowest digestibility of cell-walls. The versions with brown mid-ribs had high values for both properties and Circé was intermediate. Detailed analysis of the various morphological fractions disclosed that these genetic differences in digestibility of organic matter and of cell-wall constituents were present in all fractions except the kernel (Struik, 1982). Digestion trials in vivo on the crops of these hybrids have fully confirmed the data in vitro (Deinum et al., 1984).
Crops

Crops are communities of plants, usually of one variety. Their development, production and quality may be affected by environmental conditions and cultural practices. Struik (1983b) found that long periods of reduced irradiance reduced ear growth and development more than stem growth and also depressed digestibility of organic matter. Short periods of low irradiance were especially harmful to grain setting and digestibility when applied during flowering. High temperature stimulates development and reduces digestibility, particularly when applied during stem growth and development, when most of the cell walls are being formed (Struik et al., 1985). Cultural practices, such as high plant density, increase yield of cell wall and consequently reduce digestibility. Late sowing usually stimulates vegetative growth because temperature is higher later in spring, and postpones reproductive development. Therefore digestibility of the final crop is lower (Table 4). Struik noted differences in the digestibility of maize between fields (Table 6). The samples of fresh maize originated from a factor analysis. Soil factors had little influence on digestibility in this analysis. Differences originated partly from choice of hybrid and cultural practice but much of the small variation remained unexplained.

Table 5. Dry-matter content (% dm), apparent digestibility of organic matter ($D_{om}$), cell-wall content (% cwc), and cell-wall digestibility ($D_{cwc}$) of the whole crop for five genotypes (Struik, 1982).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Eta Ipho</th>
<th>Circé</th>
<th>INRA 240 bm3</th>
<th>INRA 188 bm3</th>
<th>LG11 bm3</th>
</tr>
</thead>
<tbody>
<tr>
<td>% dm</td>
<td>34.8</td>
<td>29.3</td>
<td>32.7</td>
<td>33.3</td>
<td>38.8</td>
</tr>
<tr>
<td>% cwc</td>
<td>38.5</td>
<td>40.4</td>
<td>41.7</td>
<td>33.2</td>
<td>36.6</td>
</tr>
<tr>
<td>$D_{om}$</td>
<td>71.8</td>
<td>75.4</td>
<td>79.8</td>
<td>81.4</td>
<td>81.8</td>
</tr>
<tr>
<td>$D_{cwc}$</td>
<td>62.6</td>
<td>70.7</td>
<td>81.1</td>
<td>80.4</td>
<td>83.0</td>
</tr>
</tbody>
</table>

Fig. 4. Correlation coefficients between the different relevant components of maize hybrids in 1978.
Table 6. Apparent digestibility (%D$_{om}$) ± standard deviation in ensiled and fresh maize samples from farmers' lots. Silage samples were representative for the Netherlands. Fresh maize came from the eastern part of the Province of Overijssel.

<table>
<thead>
<tr>
<th></th>
<th>1981</th>
<th></th>
<th>1982</th>
<th></th>
<th>1984</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%D$_{om}$</td>
<td>n</td>
<td>%D$_{om}$</td>
<td>n</td>
</tr>
<tr>
<td>Ensiled</td>
<td>25</td>
<td>73.5 ± 1.26</td>
<td>50</td>
<td>73.5 ± 1.21</td>
<td>115</td>
</tr>
<tr>
<td>Fresh</td>
<td>54</td>
<td>72.8 ± 1.39</td>
<td>54</td>
<td>73.3 ± 1.69</td>
<td></td>
</tr>
</tbody>
</table>

Hybrids differ in their response to late sowing, high plant density and other cultural practices. This response has been investigated extensively for grain yield and whole-plant yield. However, a response is also seen in digestibility, dry-matter content and cell-wall content (Phipps, 1980; Struik, 1983a). Phipps (1980) even advocated the use of prolific lines in breeding forage maize. The relevance of differences between hybrids in their response to cultural practice for quality characters needs further evaluation. In our view, however, these differences in hybrid response are less important than genetic differences in cell-wall digestibility.

Our data indicate that the interaction genotype × location and the interaction genotype × year (Deinum & Bakker, 1981; Beerepoot, 1981) are low for digestibility and cell-wall content of forage maize. This is in agreement with observations on other crops such as forage sorghum (Pedersen et al., 1982) and switchgrass (Panicum virgatum L.) (Vogel et al., 1981). Extensive information on digestibility of forage maize and the interactions hybrid × environment and hybrid × cultural practice will become available in the near future from the FAO subnetwork on 'The nutritive value of the whole maize plant', in which several European institutes are participating.

Regions and climates

The extensive research carried out in the Netherlands during the last ten years has shown that the average digestibility of organic matter of fresh and ensiled maize was about 73.5% (Table 6). The main hybrids were LG11, Fronica and Dorina. The low digestibility in 1984 could be ascribed to the lack of sunshine and of photosynthesis during grain filling.

Research reported in the literature reveals that there are appreciable differences in digestibility between regions and climates (Table 7). These differences may be caused by several

Table 7. Average digestibility of maize silage in various countries.

<table>
<thead>
<tr>
<th>Country</th>
<th>Digestibility (%)</th>
<th>To appetite/restricted</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Netherlands</td>
<td>73.5 (D$_{om}$)¹</td>
<td>restr.</td>
<td>Deinum et al., 1984</td>
</tr>
<tr>
<td>Belgium</td>
<td>73 (D$_{om}$)</td>
<td>restr.</td>
<td>Aerts et al., 1978</td>
</tr>
<tr>
<td>West Germany</td>
<td>68.8 (D$_{om}$)²</td>
<td>restr.</td>
<td>Gross &amp; Averdunk, 1974</td>
</tr>
<tr>
<td>France</td>
<td>70.4 (D$_{om}$)</td>
<td>appet.</td>
<td>Andrieu, 1985</td>
</tr>
<tr>
<td>United States</td>
<td>65.3 (D$_{om}$)²</td>
<td>appet.</td>
<td>Goering et al., 1969</td>
</tr>
<tr>
<td>United States</td>
<td>64.0 (D$_{om}$)</td>
<td>appet.</td>
<td>Schmid et al., 1975</td>
</tr>
</tbody>
</table>

1. Digestibility of organic matter.
2. Digestibility of dry matter.

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factors: choice of hybrid, growing conditions and procedure for digestibility trials. In the Netherlands, digestibility is measured in vivo under standard conditions at maintenance level with wethers supplied with sufficient protein. In other institutes, digestibility may be measured for intake to appetite, without protein supplementation, or in cows. Such differences in procedures may account for digestibility differences of 2-3 percentage units. Growing conditions (plant density, irradiance, temperature) can deviate from those in the Netherlands. Temperature has the greatest influence and according to our research a temperature 10 °C higher may cause a digestibility of organic matter 3 percentage units lower (Struik et al., 1985).

The remaining factor that could account for differences in digestibility between regions and climates is the average influence of genetic characters of the hybrids between regions. These genetic characters may easily account for a digestibility difference of 5 percentage units, and we may assume that the relatively low digestibilities of maize in West Germany and the United States partly result from the particular hybrids chosen in those countries.

Prospects

Substantial genetic variation in digestibility has been found under Dutch growing conditions (Deinum & Bakker, 1981). The older, better digestible, hybrids had some inbred lines in common, like F2 and F7. The newer, more productive but less digestible hybrids have some other inbred lines in common.

Beerepoot (1981) studied the heritability of digestibility in single cross hybrids in a trial with 11 dent x 13 flint inbred lines. The digestibility of organic matter of stover ranged from 54.9 to 65.2% and digestibility of organic matter of whole crop ranged from 73.3 to 78.3%. The lowest digestibility was similar to that of Eta Ipho, which was the lowest of the standards in the trials. Narrow-sense heritability for digestibility of organic matter in this trial was 0.71. As far as we could ascertain, no genetic analysis of properly measured digestibilities has been published for forage maize. In other forage grasses, digestibility has been found to vary substantially and to be highly heritable; for example Dangi et al. (1979) and Pedersen et al. (1982) reported large GCA values in forage sorghum.

Beerepoot (1981) also studied the correlation of digestibility of organic matter with all kinds of factors to discover whether there are visible non-destructive criteria for digestibility (Table 8). There was a slight negative correlation between yield and digestibility. Unfortunately, no character was found that correlated closely with digestibility. At present, attempts are being made to investigate whether techniques such as near-infrared reflectance spectroscopy and

<table>
<thead>
<tr>
<th>Trait</th>
<th>Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digestibility of the crop ~ % ear</td>
<td>0.69</td>
</tr>
<tr>
<td>Digestibility of the crop ~ Dig. stover</td>
<td>0.56</td>
</tr>
<tr>
<td>Digestibility of the crop ~ % dm</td>
<td>0.38</td>
</tr>
<tr>
<td>Digestibility of the crop ~ date of 50% silking</td>
<td>-0.27</td>
</tr>
<tr>
<td>Digestibility of the crop ~ stem Ø</td>
<td>-0.30</td>
</tr>
<tr>
<td>Digestibility of the crop ~ plant length</td>
<td>-0.68</td>
</tr>
<tr>
<td>Digestibility of stover ~ % dm crop</td>
<td>-0.23</td>
</tr>
<tr>
<td>Digestibility of hybrid ~ Dig. parents</td>
<td>0.50</td>
</tr>
<tr>
<td>Digestibility of the crop ~ crop yield</td>
<td>-0.29</td>
</tr>
</tbody>
</table>
cellulose digestion correlate sufficiently well with digestibility to enable them to be used in screening.

None of the hybrids studied by Beerepoot showed brown mid-ribs, indicating that other genes control digestibility as well. There is sufficient unexploited genetic variation to be able to obtain digestibilities as high as those of mutants with brown mid-ribs without recourse to bm genes and so perhaps without their agronomic disadvantages.

Cooperative research recently started in different departments of the Agricultural University in Wageningen to detect the origin of genetic variation in digestibility of cell walls. We are studying the histological composition of the various morphological fractions, including the rate and extent of digestibility of various tissues, and ultrastructure of cell walls.

The discovery of varietal differences in digestibility has led to digestibility being adopted as a criterion in varietal testing. The relative performance of the hybrids for yield of dry matter and nutritive value has been mentioned since 1983 in the list of recommended varieties (Table 9). Recently introduced varieties are, in general, less digestible than those introduced in the late 1960s and early 1970s (Figure 5) and it is hoped that this downward trend can soon be reversed.

![Graph showing relative digestibility of organic matter (%D_{om})](image)

**Fig. 5.** Reduction in relative digestibility of organic matter (%D_{om}) resulting from the introduction of new varieties (Struik, 1983a).

**Table 9.** Relative performance in yield of dry matter and of nutritive value of some maize varieties mentioned in the 60th Descriptive List of Varieties of Agricultural Crops, 1985.

<table>
<thead>
<tr>
<th>Hybrid</th>
<th>Year of acceptance</th>
<th>Relative yield (%)</th>
<th>dry matter</th>
<th>digestible organic matter</th>
</tr>
</thead>
<tbody>
<tr>
<td>LG11</td>
<td>1972</td>
<td>94</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>Brutus</td>
<td>1980</td>
<td>96</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td>Dorina</td>
<td>1980</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Splenda</td>
<td>1983</td>
<td>103</td>
<td>103</td>
<td></td>
</tr>
<tr>
<td>Markant</td>
<td>1983</td>
<td>103</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Clipper</td>
<td>1985</td>
<td>103</td>
<td>101</td>
<td></td>
</tr>
</tbody>
</table>
In a small country with rather uniform growing conditions like the Netherlands, the variation in digestibility of 'normal' forage-maize crops is very small. Therefore we should be able to predict digestibility annually from the weather experienced during growth. Then the farmer has only to correct for hybrid choice.

In the introduction, it was stated that plant characteristics affect digestibility, intake and feed efficiency. These qualitative factors are interrelated, especially when the forage is fed to highly productive livestock. Ideally, as Gallais et al. (1980) clearly showed, trials in vivo should use high intakes for proper evaluation of the quality of a genotype. Unfortunately, this is impossible for the large number of samples in a breeding programme. Digestibility in vitro is commonly accepted as the best approach for evaluation of forage quality. However the significance of this method for establishing genetic differences in quality of feed for highly productive cattle needs further assessment.

As digestibility has become a criterion in varietal testing, we may expect hybrids with improved digestibility in other countries too. Even greater improvements may be expected if the investigations into the fundamental aspects of cell-wall development and cell-wall digestion yield promising results and if these results can be exploited in the breeding and husbandry of forage maize. Then, the net energy intake by livestock of forage maize may approach that of concentrates.

References


Deinum, B., 1976. Effect of leaf age, leaf number and temperature on cell-wall and digestibility of maize.


Improving the nutritive value of maize

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Summary

To find suitable selection criteria for an efficient breeding strategy for silage maize quality in four experiments, different cultivars, lines and single crosses from a diallele were tested on correlation between cellwall constituents and digestibility. The following results were obtained.

All maize cultivars examined, lines and single crosses from a diallele showed wide genetic variation in cell-wall constituents and in digestibility of stover. As a working hypothesis, we may distinguish the following types: (a) types that differ in value and range of lignin content and digestibility of stover, indicated by lignin content; (b) types with different decrease in digestibility. This effect was genetically linked with a higher value and bigger differences for lignin content and for digestibility between the cultivars at the site more favourable for maize production. The reason for the different decreases in digestibility is a different structure of cell-walls.

Descriptors: silage maize, Zea mays L., digestibility, breeding, cell-wall, heritability

Introduction

Whole-crop silage maize attracts farmers
- by its high content of energy for lactation (6.5 - 6.8 MJ/kg) achieved by high digestibility of organic matter (>73%)
- by reaching a dry-matter content at maturity of 300 g/kg or more (Zimmer, 1981).
If both conditions are met, we achieve
- a minimum in conservation losses
- a maximum in feed intake.

Today maize varieties of FAO maturity rating 200 - 250 (early or medium-early) reach satisfactory quality even in north-west Germany, as a result of continuous improvement (Table 1).

On the farm, quality traits have reached reasonable stability (Table 2).

Working hypothesis

To improve the nutritive value under suboptimal climatic conditions, which do not favour grain-filling, the following ideas have been brought together and tested for several years (Collaboration with W.G. Pollmer, University Hohenheim, and F. Gross, Bayerische Landesanstalt für Tierzucht, Grub).
- The stover contributes a larger proportion of its digestible lignocellulose to total energy yield.
Table 1. Development of maize characteristics (FAO maturity rating 200-250) (performance trials 1956-1984; \( n = 27 \) years). Data from Berichte Bundessortenamt, Ergebnisse der Wertprüfung mit Silomais, Sortiment früh - mittelfrüh.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Northern Germany</th>
<th>Central - Southern Germany</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1950s today</td>
<td>1950s today</td>
</tr>
<tr>
<td>content of DM in whole crop (g/kg)</td>
<td>219 305</td>
<td>208 349</td>
</tr>
<tr>
<td>increment per year</td>
<td>+ 2.97 ± 1.42</td>
<td>+ 4.84 ± 0.63</td>
</tr>
<tr>
<td>yield of DM from whole crop (t/ha)</td>
<td>10.08 17.93</td>
<td>10.55 18.02</td>
</tr>
<tr>
<td>increment per year</td>
<td>+ 0.027 ± 0.029</td>
<td>+ 0.257 ± 0.033</td>
</tr>
<tr>
<td>content of ear DM in total dry matter (g/kg)</td>
<td>413 483</td>
<td>420 503</td>
</tr>
<tr>
<td>increment per year</td>
<td>+ 2.41</td>
<td>+ 2.86</td>
</tr>
<tr>
<td>digestibility of whole-crop DM (%)</td>
<td>69.9 71.2</td>
<td>69.7 71.9</td>
</tr>
<tr>
<td>content of net energy for lactation in DM (MJ/kg)</td>
<td>5.89 6.30</td>
<td>5.84 6.50</td>
</tr>
<tr>
<td>Starch equivalent (g/kg)</td>
<td>579 613</td>
<td>575 630</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Fresh</th>
<th>Silage</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>content of DM (g/kg)</td>
<td>( \bar{x} \pm s )</td>
<td>( \bar{x} \pm s )</td>
<td>( s ) between years &gt; ( s ) within years</td>
</tr>
<tr>
<td>trend over 14 years (g kg(^{-1}) year(^{-1}))</td>
<td>+ 6.5</td>
<td>( s ) within years &gt; ( s ) between years</td>
<td></td>
</tr>
<tr>
<td>digestibility of organic matter (%)</td>
<td>74.0 ± 1.1</td>
<td>69.1 ± 2.5</td>
<td>( no ) trend</td>
</tr>
<tr>
<td>content of net energy for lactation (MJ/kg)</td>
<td>6.81 ± 0.14</td>
<td>6.25 ± 0.15</td>
<td>( s ) within years &gt; ( s ) between years</td>
</tr>
</tbody>
</table>

- The compensation between ears and stover should be evaluated, assuming a constant energy content in the whole crop.
- Genetic variability of organic-matter digestibility of stover is known; on the other hand, the net energy content of ears over a wide range remains rather constant (8.3 MJ/kg); relative standard deviation <0.05; Gross & Peschke, 1980).
- From experimental data, it became obvious that a change in the content of ear of about 50 g/kg and a change in content of acid-detergent fibre of 10 g/kg have the same effect on the digestibility of the crop (Zimmer, 1981).
  
  As a consequence,
- an efficient breeding strategy for quality has to rely on suitable selection criteria; here the cell-wall parameter and digestibility of the stover are of greatest importance
- furthermore, only efficient methods for quality assessment will help breeders to follow such a strategy in future.
Results

**Yield structure and quality** Digestibility of organic matter is a major parameter of quality. With the Tilley & Terry (1963) method, we have an excellent assay, though inefficient for breeding purposes. Cell-wall parameters, such as contents of neutral detergent fibre (cell-wall constituents), acid-detergent fibre and acid-detergent lignin are, however, directly related to digestibility. In animal nutrition, feeds of different species, origin and stage of maturity fit into this basic correlation. The question still needs a better answer whether genetic variability can be analysed and predicted precisely enough and how (Wermke & Theune, 1980; Zimmer et al., 1980).

In cooperation with a German maize breeder, a trifactorial trial with 12 varieties at 7 sites, further denoted as Experiment 1, was run, to analyse relevant data on yield and quality (Wermke & Theune, 1980; Zimmer et al., 1980; Wermke & Zimmer, 1982). The main results are given in Table 3.

- Genetic variance of cell-wall traits was in the range 1.08 - 3.26%. There was a high interrelation of year and site.
- The heritability of those traits was 0.46 - 0.86.
- Digestibility of organic matter could be estimated with the following polyfactorial regression, where the most important traits are lignin and ash, with an obvious influence of the particular stage of ear development.


<table>
<thead>
<tr>
<th>Characteristic of stover</th>
<th>Mean</th>
<th>Variance (%)</th>
<th>Heritability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>variety</td>
<td>site</td>
</tr>
<tr>
<td>DM yield (t/ha)</td>
<td>8.69</td>
<td>7.8</td>
<td>3.8</td>
</tr>
<tr>
<td>DM content (g/kg)</td>
<td>227</td>
<td>5.6</td>
<td>8.7</td>
</tr>
<tr>
<td>cellular contents in DM (g/kg)</td>
<td>309</td>
<td>3.9</td>
<td>9.9</td>
</tr>
<tr>
<td>content of cell-wall constituents in DM (g/kg)</td>
<td>614</td>
<td>1.7</td>
<td>4.3</td>
</tr>
<tr>
<td>content of acid-detergent fibre in DM (g/kg)</td>
<td>338</td>
<td>1.1</td>
<td>4.5</td>
</tr>
<tr>
<td>content of acid-detergent lignin in DM (g/kg)</td>
<td>32</td>
<td>3.3</td>
<td>6.4</td>
</tr>
<tr>
<td>cellulose content (ADF-ADL) in DM (g/kg)</td>
<td>306</td>
<td>1.4</td>
<td>4.4</td>
</tr>
<tr>
<td>hemicellulose content (NDF-ADF) in DM (g/kg)</td>
<td>276</td>
<td>2.4</td>
<td>4.0</td>
</tr>
<tr>
<td>organic matter digestibility in vitro (%)</td>
<td>63.9</td>
<td>1.5</td>
<td>4.0</td>
</tr>
<tr>
<td>content of net energy for lactation in DM (MJ/kg)</td>
<td>6.33</td>
<td>1.4</td>
<td>3.4</td>
</tr>
<tr>
<td>content of ears in DM (g/kg)</td>
<td>481</td>
<td>4.9</td>
<td>5.7</td>
</tr>
</tbody>
</table>
\[ \text{digestibility of organic matter} = 110.07 - 3.367 \times \text{content of acid-detergent lignin} - 0.301 \times \text{content of acid-detergent fibre} - 0.904 \times \text{ash content} - 0.227 \times \text{content of DM in ears} - 0.128 \times \text{proportion of ears} - 0.053 \times \text{carbohydrates} \]

\[ r^2 = 0.641; n = 239 \]

Furthermore different growth types, i.e. Type A and B were distinguished (Zimmer & Wermke, 1983; Struik, 1982) (Table 4), showing that with high dry-matter yield, differences in balance between ear content and stover quality end up with the same yield and content of net energy. Translocation in the grain-filling phase is typical for Type A; it reduces assimilation activity. In contrast, Type B continues assimilation in stover, and reaches the same stage of maturity in the ears at harvest time.

Table 4. Performance trial with forage maize (12 cvs × 6 sites × 3 years).

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Type A</th>
<th>Type B</th>
<th>Diff.</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM yield (t/ha)</td>
<td>16.62</td>
<td>17.39</td>
<td>17.55</td>
<td></td>
</tr>
<tr>
<td>ear content in total DM (g/kg)</td>
<td>478</td>
<td>504</td>
<td>431</td>
<td>- 73</td>
</tr>
<tr>
<td>digestibility of stover organic matter (%)</td>
<td>64.9</td>
<td>62.4</td>
<td>64.7</td>
<td>+ 2.3</td>
</tr>
<tr>
<td>yield of digestible DM (t/ha)</td>
<td>11.32</td>
<td>11.86</td>
<td>11.96</td>
<td></td>
</tr>
<tr>
<td>yield of net energy for lactation (GJ/ha)</td>
<td>120.4</td>
<td>124.9</td>
<td>125.5</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Changes in DM yield of different types of maize with time.
Yield of DM (Fig. 1) and cell-wall composition (Fig. 2) of both types of variety have been studied for 2 years (Experiment 2). The results indicate:

- Type A developed ears mainly by translocation; stover digestibility and cellular content swiftly decreased, but content of cell-wall constituents increased by translocation to the ear.
- Type B continued assimilation markedly, continuing to increase yield of stover while keep-
ing its composition almost constant at a higher digestibility of organic matter because of less translocation to the ear.

Genotype and cell-wall traits  Our studies and data from other authors confirm that similar or even the same chemical composition of the cell-wall constituents is not necessarily associated with the same digestibility. So plant breeders must look for interesting genotypes by means of the correlation between cell-wall content and digestibility of organic matter. The following results are illustrative. Figure 3 compares composition and digestibility of stover. Cell-wall digestibility differs, indicating a different physical structure restricting activity of enzymes. Figure 4 shows the effect of lignification (the ratio of acid-detergent lignin to dry matter) over time, where differentiation of stover quality at a younger stage is much higher.

From that, we examined cell-wall characteristics of lines for breeding purposes. We obtained four sets of data from different sites and years (Exp. 3 at Völkenrode in 1981 and 1983, and at Eckartsweier in 1982 and 1984). Content of acid-detergent lignin proved to be the best independent variable for prediction of digestibility (Fig. 5). Despite environmental effects the lines proved always to be in the same order. The 'best' and 'worst' lines showed stable differences in content of proximate components in dry matter (Table 5). There were significant differences in the rate of lignification.

A diallele was formed at Eckartsweier based on the available information and has been multiplied since 1983. Already it is giving interesting results (Fig. 6). For digestibility as a function of lignin content, data from 12 parental lines are scattered to the extent already known from Figure 6 or Table 5. Grouping of the 10 best and the 10 worst single crosses shows up distinct differences in composition and digestibility (Fig. 7). Clear compensation is shown

\[ y = 89.98 - 0.7744x \]
\[ r^2 = 0.72^{**} \]

\[ y = 76.54 - 0.4996x \]
\[ r^2 = 0.49^* \]

Fig. 4. Relation of content of acid-detergent lignin (ADL) in dry matter to digestibility of organic matter in stover of different types of maize.
Table 5. Maize stover composition and digestibility (12 lines, 4 data sets; Eckartsweier, 1981, 1983; Völkkenrode, 1982, 1984). (Hemicellulose was calculated as cell-wall constituents minus acid-detergent lignin; cellulose as acid-detergent fibre minus acid-detergent lignin.)

<table>
<thead>
<tr>
<th></th>
<th>Mean 3 'best'</th>
<th>Mean 3 'worst'</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 36</td>
<td>n = 12</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Mean 3 'best'</th>
<th>Mean 3 'worst'</th>
</tr>
</thead>
<tbody>
<tr>
<td>digestibility of organic matter</td>
<td>0.618</td>
<td>0.671</td>
<td>0.565</td>
</tr>
<tr>
<td>content of ash (g/kg)</td>
<td>75</td>
<td>69</td>
<td>76</td>
</tr>
<tr>
<td>cellular content (g/kg)</td>
<td>275</td>
<td>313</td>
<td>247</td>
</tr>
<tr>
<td>content of cell-wall constituents (g/kg)</td>
<td>652</td>
<td>618</td>
<td>677</td>
</tr>
<tr>
<td>content of hemicellulose (g/kg)</td>
<td>308</td>
<td>297</td>
<td>312</td>
</tr>
<tr>
<td>content of cellulose (g/kg)</td>
<td>309</td>
<td>291</td>
<td>324</td>
</tr>
<tr>
<td>content of acid-detergent lignin (g/kg)</td>
<td>35</td>
<td>38</td>
<td>41</td>
</tr>
<tr>
<td>digestibility of cell-wall constituents (%)</td>
<td>47.0</td>
<td>51.4</td>
<td>41.5</td>
</tr>
</tbody>
</table>

from the data on ear content and stover quality of the 10 worst single crosses (Fig. 7). From pedigree, the best and worst lines have a disproportionate share in the groups of best or worst single crosses. Differences in stover quality between these groups can again be explained by prolonged activity in the stover, by a difference in physiological age and by differences in cell-wall structure and digestibility.

**Time of sampling** If material is to be screened for stover quality, time of evaluation needs attention, since lignification and therefore digestibility will follow different time courses. As a
Fig. 6. Correlation between digestibility of organic matter (OMD) and content of acid-detergent lignin (ADL) in dry matter for 12 lines (O; I-IX, X, Y, Z), 10 single crosses with worst digestibility (▲) and 10 single crosses with best digestibility (●).

\[ y = 78.978 - 4.869x \]
\[ r^2 = 0.636*** \]
\[ N = 168 \]

Fig. 7. Content in dry matter and digestibility of proximate components of stover from different lines of maize from the Eckartsweier diallele.
working hypothesis, we may distinguish the following types, which also differ statistically in quality traits.
- Types with different lignin content
  The two types (Fig. 8A) differ in value and range of lignin content and digestibility of stover. The breeder can quickly and simply recognize the different types by analysis of lignin content or digestibility at a similar phenological stage (as indicated by lignin content or by digestibility).
- Types with different decrease in digestibility
  The regression (Fig. 8B) of the 10 worst single crosses has a less steep regression for digestibility at their lower values than those of the 10 best. For correct critical examination, the breeder needs to analyse lignin content and digestibility at two phenological stages.

**Phenological effect on correlation between lignin content and digestibility** The regression function (Fig. 8C) for both cultivars at both sites shows a parallel decrease. This was genetically linked with a higher value and bigger differences for lignin content and for digestibility between the cultivars at the site more favourable for maize production. The breeder needs to know lignin content and digestibility at two phenological stages and at two sites to assess any breeding material. The reason for the different decreases in digestibility is a different structure of cell-walls, because digestibility decreases more in lines with a negative correlation between digestibility and cellulose content and less in the line with a positive correlation (Table 6).

**Conclusions**

The general function for digestibility of organic matter with acid-detergent fibre, acid-detergent lignin reveals that a lot of maize cultivars show a very strong correlation between cell-wall constituents and digestibility of organic matter. It also includes a wide genetic variability (Exp. 1 and 2).

Genotypes differ in value and in decrease in digestibility with maturation. There are
Table 6. Relationship between digestibility and content of cell-wall constituents and of cellular contents in stover dry matter of 3 lines of silage maize (Eckartsweier, 1982). (Variation of the independent variable as a proportion (%) of total variation of the independent variable) (coefficient of determination $r^2$).

<table>
<thead>
<tr>
<th>Relation</th>
<th>Line E</th>
<th>Line F</th>
<th>Line G</th>
</tr>
</thead>
<tbody>
<tr>
<td>lignin content $\times$ digestibility</td>
<td>$(-0.878)$</td>
<td>$(-0.713)$</td>
<td>-</td>
</tr>
<tr>
<td>cellulose content $\times$ digestibility</td>
<td>$(-0.099)$</td>
<td>$(+0.170)$</td>
<td>$(-0.994)$</td>
</tr>
<tr>
<td>hemicellulose content $\times$ digestibility</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>cellular content $\times$ digestibility</td>
<td>$(-0.018)$</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>total $\times$ digestibility</td>
<td>$0.995$</td>
<td>$0.883$</td>
<td>$0.994$</td>
</tr>
<tr>
<td>lignin content (g/kg)</td>
<td>295</td>
<td>339</td>
<td>322</td>
</tr>
<tr>
<td>digestibility of organic matter (%)</td>
<td>61.4</td>
<td>60.0</td>
<td>60.3</td>
</tr>
<tr>
<td>average</td>
<td>67.7</td>
<td>63.7</td>
<td>70.4</td>
</tr>
<tr>
<td>first stage</td>
<td>57.4</td>
<td>59.4</td>
<td>54.9</td>
</tr>
<tr>
<td>fifth stage</td>
<td>-10.3</td>
<td>-4.3</td>
<td>-15.5</td>
</tr>
<tr>
<td>change in digestibility</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Cultivars with high digestibility in an earlier growth stage with fast decrease and other cultivars with low digestibility during the earlier growth stages with a slower decrease.

If material is sampled between milk and dough ripeness it is containing 25 - 35 g/kg acid-detergent lignin, differences between cultivars become smaller at a later stage of maturity.

Chemical composition was similar or only slightly different (Fig. 5 and 7). However, there were large differences in digestibility of organic matter and of cell-wall material attributable to physical structure.

References


Stover forage quality as affected by stalk strength in maize (Zea mays L.)

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2. Department of Agronomy, Iowa State University, Ames, Iowa, 50011, USA

Summary

The primary objectives of this study were to evaluate the effect of stalk strength on quality of stover as forage, and to relate dry-matter yield of stover and grain yield to stalk strength, male sterility, and plant density.

Seven entries with a wide range of stalk strength were grown at two sites: male-sterile with ears covered (MSC); normal counterpart (N); male sterile with ears exposed (MSU); prolific hybrid (PR); and the zero (C₀), first (C₁), and third (C₃) cycles of selection for resistance to stalk rot from a synthetic variety. They were planted at three plant densities of about 32 000, 64 000 and 96 000 ha⁻¹ (3.2, 6.4 and 9.6 m⁻²).

As measures of forage quality were taken content of acid-detergent fibre (ADF), acid-detergent lignin (ADL) and dry matter disappearance in vitro (IVDMD). Their values for whole internode, rind, pith and stover were not adversely affected by stalk strength. Stover yield was greatest in the male-sterile covered and was least in the prolific hybrid. There was no clear loss of grain yield with increasing stalk strength, and male sterility was responsible for the greatest yield under stress. The prolific hybrid also showed relatively high yields.

Descriptors: maize, Zea mays L., stover, forage quality, stalk strength, breeding, disease resistance

Introduction

Lodging in maize has become a greater problem as farmers have increased the use of nitrogen fertilizer, have raised plant densities, and have increasingly mechanized harvesting. Even though plant breeders have improved resistance to lodging, stalk lodging continues a problem.

Plant breeders' contributions have been mostly to grain yield. They have disregarded the effects that lodging resistance could have on forage quality. Histological studies indicate that strong stalks in hybrids would be expected to contain more cell-wall material and would therefore be less digestible (Hunter & Dalbey, 1937; Magee, 1948; Murdy, 1960). Greater contents of lignin and cellulose have often been reported as associated with lodging resistance (Pinthus, 1973). These structural materials would be expected to decrease forage quality. A recent study, however, reported that stalk strength seemed to be associated with improved forage quality (Albrecht et al., 1983).

It has been shown that increasing stalk strength generally causes a decrease in grain yield (Thompson, 1982; Davis & Crane, 1976; Albrecht et al., 1983). There are also reports that cytoplasmic male-sterility can increase yield of grain and dry matter yield of high-sugar forage especially under stress, for instance high plant density, low soil moisture, and poor soil fertility (Chinwuba et al., 1961; Meyer, 1970; Bruce et al., 1966; Buren, 1970).
Materials and methods

The trial was conducted at two sites (Ames and Nashua, Iowa, United States) in 1983. Seven entries were tested with a wide range of stalk strength: male-sterile covered (MSC); normal counterpart (N); male-sterile exposed (MSU); prolific hybrid (PR); and the zero (C₀), first (C₁), and third (C₃) cycles of selection for resistance to stalk rot from a synthetic variety (Iowa BS1). They were planted at three plant densities of about 32 000, 64 000 and 96 000 ha⁻¹ (3.2, 6.4 and 9.6 m²). Two harvests were taken, the first at mid-silking and the second at physiological maturity.

The trial was of split-split plot design with three replicates. Plant densities were established as the whole plots, seven entries as the subplots, and two harvests as the subsubplots. Each subplot consisted of four rows 9.90 m long and 0.76 m apart.

Stalk strength was measured with a machine and method described by Durrel (1925), and modified by Jenkins (1930) and Jenkins & Gaessler (1934). All the stalks were tested at the second elongated internode, with force applied in the middle of the minor diameter. Stalk strength was calculated as loading divided by stalk area.

Content of acid-detergent fibre (ADF), acid-detergent lignin (ADL), and dry matter disappearance in vitro (IVDMD) were estimated for the whole internode, rind, and pith on portions of the stalks. These portions were halves of the second elongated internode plus half of each adjacent internode. Rind and pith were separated by scraping the pith from the rind with a spatula, dried at 60 °C for further analysis, and ground to pass a 1-mm screen. The IVDMD of stover was also determined. Grain yield and stover yield were also estimated.

Content of ADL and ADF were determined by methods of Goering & Van Soest (1970), with procedural modifications as proposed by Van Soest & Robertson (1980). Disappearance of dry matter was determined by a direct-acidification two-stage procedure (Marten & Barnes, 1980). Grain yield was evaluated in the two middle rows of each plot. Stover yield was determined after picking the ears in 2.10 m of one of the middle rows. Subsamples of stover, after being chopped, as well as of ears were taken for estimation of dry-matter. Subsamples were placed in nylon net bags and dried at 65 °C to constant weight in a forced-air drier.

Results and discussion

Stalk strength decreased significantly with increasing plant densities (Table 1). Although the interaction of plant density and entry was highly significant, examining each entry by plant density indicated that stalk strength did not decrease significantly with plant density (from 64 000 to 96 000 ha⁻¹ (6.4 to 9.6 m²) except in the male-sterile covered hybrid (MSC). The

<table>
<thead>
<tr>
<th>Plant density (m²)</th>
<th>Entry</th>
<th>C₁</th>
<th>C₀</th>
<th>N</th>
<th>MSU</th>
<th>PR</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2</td>
<td>MSC</td>
<td>1.29</td>
<td>0.94</td>
<td>0.66</td>
<td>0.61</td>
<td>0.88</td>
</tr>
<tr>
<td>6.4</td>
<td></td>
<td>1.14</td>
<td>0.70</td>
<td>0.60</td>
<td>0.54</td>
<td>0.75</td>
</tr>
<tr>
<td>9.6</td>
<td></td>
<td>0.90</td>
<td>0.68</td>
<td>0.56</td>
<td>0.55</td>
<td>0.71</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>1.11</td>
<td>0.77</td>
<td>0.61</td>
<td>0.57</td>
<td>0.77</td>
</tr>
</tbody>
</table>

Table 1. Stalk strength (MPa) in relation to plant density and entry. Least significant differences (P<0.05) were for plant density 45 kPa, entry 56 kPa and for plant density at same or different entry 107 kPa.
entry from the first cycle of selection was not affected by plant density. The overall entries means indicate that male-sterile covered was the strongest (1.1 MPa). The third cycle of selection (C₃), normal (N), and male-sterile exposed (MSU) were intermediate, and synthetic variety (C₀), first cycle (C₁), and prolific (PR) were the weakest.

Greatest contents of acid-detergent fibre were found in the male-sterile uncovered and the prolific (Figure 1). The male-sterile covered showed the lowest contents for whole internode, rind, and pith, respectively, 334, 466 and 145 g/kg.

The prolific hybrid (PR), which was one of the weakest, had the highest content of acid-detergent lignin in internode, rind, and pith (Figure 2). The male-sterile covered hybrid

![Content of acid-detergent fibre in dry matter (g·kg⁻¹)](image1)

**Fig. 1.** Content of acid-detergent fibre in whole internode, rind and pith in relation to entry.

![Content of acid-detergent lignin (g·kg⁻¹)](image2)

**Fig. 2.** Content of acid-detergent lignin in whole internode, rind and pith in relation to entry.
(MSC), which was the strongest, showed the lowest value, especially for whole internode and pith.

Disappearance of dry-matter for the whole internode, rind, and pith was greatest in the strongest hybrid such as male-sterile covered (MSC) and least in one of the weakest-stalk hybrids such as the prolific (PR, Figure 3). There were similar trends for stover. So stover of the prolific hybrid had the poorest quality as feed.

Yield of stover dry-matter was greatest in the male-sterile covered (MSC) and least in the prolific (PR) hybrid (Figure 4). Average grain yield was reduced by increasing plant density for most of the hybrids. This occurred because the 1983 season had weather extremes, both of

![Fig. 3. Digestibility of dry matter in vitro for whole internode, rind, pith and stover in relation to entry.](image)

![Fig. 4. Yield of stover dry matter in relation to entry.](image)
rainfall and temperature. Even though the spring was wet, the maize plants suffered some moisture stress in July and August, especially at Nashua, with temperature stress at both sites. The prolific hybrid, though, was not significantly affected, nor was the male-sterile uncovered (Figure 5).

Conclusions

The results of this study show that the quality of the whole internode, rind, pith, and stover was not adversely affected by increasing stalk strength. In fact, content of ADF and ADL decreased in the stronger-stalked hybrids, whereas IVDMD increased. The prolific hybrid had the least stover quality.

Stover yield was greatest in the male-sterile covered hybrid and was least in the prolific hybrid. There was no clear loss of grain yield with increasing stalk strength, and male-sterility was responsible for greatest yields under stress. The prolific hybrid also produced relatively high yields.

References


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Stalk strength in maize with brown mid-ribs

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Summary

In selection for increase in resistance to lodging of \( bm_3 \) genotype, we obtained 13 inbred lines that did not differ in puncture strength for penetration into stalk from a normal control. The puncture strength of \( bm_3 \) lines at full maturity was 31.96-53.54 MPa. At that stage, the control inbred MA21 had a value of 35.20 MPa. Increased stalk strength was observed also in three experimental hybrids that at milky-dough and full maturity did not differ in puncture strength from the standard hybrid. Lignin content in vegetative dry-matter of \( bm_3 \) hybrids was 77.2, 75.9 and 83.0 g/kg, and in the control 97.8 g/kg. The study showed ways of obtaining \( bm_3 \) genotypes with increased resistance to stalk lodging through recurrent selection.

Descriptors: maize, Zea mays L., brown mid-rib, lignin, stalk strength

Introduction

One way of improving the feeding value of maize forage by selection is to use \( bm \) mutants (brown mid-rib) with decreased content of lignin. Of four \( bm \) mutants, the most effective seems to be \( bm_3 \), which had the lowest lignin content (Muller et al., 1971). Decreased lignin content in this mutant could be observed by the increase in digestibility of dry matter in vitro (Lechtenberg et al., 1972), increased feeding value of silage (Teljatnikov et al., 1978) and increased feed efficiency with this silage (Gallais et al., 1980).

The study of \( bm \) mutants showed that \( bm \) gene did not influence only the structural composition, but also influenced negatively some agronomical characters of these genotypes. The main ones include decrease in lodging resistance, dry-matter yield and grain content (Zuber et al., 1977; Gallais et al., 1980; Miku, 1981). These traits do no depend only on \( bm \) gene, but also on genetic background and it seems possible to produce genotypes with low lignin content and acceptable agronomic characters (Gallais et al., 1980; Miku, 1981).

In the Maize Research Institute at Trnava, we have been working with \( bm \) gene since 1973. At the first stage, \( bm_3 \) gene was back-crossed into standard inbred lines to obtain counterparts of normal inbreds. We have produced 16 lines that are now in \( S_6 \) generation. Since these inbred lines and their hybrids showed several of the negative traits, especially stalk lodging, we started recurrent selection to increase resistance to lodging in 1977. Results of this selection are summed up in this paper.

Materials and methods

The source of \( bm_3 \) gene was the single cross W37A \( bm_3 \times W64A \) \( bm_3 \), which was crossed
with a set of 9 normal lines. After selfing, plants with \( bm_3 \) gene were detected and selfed in \( S_1 \) generation. In the next year, the progenies of selfed plants were sown in ear rows and at full maturity puncture strength of penetration into stalk was measured (Chang et al., 1976). The needle of the puncture strength indicator was stabbed in the 2nd internode of the stalk. Three mutant plants with best performance were selected from each progeny. Plants were intercrossed and hybrids were sown in bulk. The procedure was repeated also in the next year with plants that again showed good performance. After that, inbred lines were bred by standard method. The choice of plants for the next generation was based on puncture strength.

Results and discussion

Table 1 sums up 14 inbred lines that were best out of the tested set of inbred lines and did not differ in the puncture strength for penetration into the stalk from the normal check. Values for mutant inbred lines in milky-dough stage ranged from 31.18 to 50.21 MPa at this stage of maturity, 5 inbred lines reached a stalk strength of over 40 MPa: Tva-3901/1 \( bm_3 \); Tva-3902 \( bm_3 \); Tva-3903 \( bm_3 \); Tva-3907/1 \( bm_3 \); Tva-3907/2 \( bm_3 \). These inbred lines differed statistically from the control inbred MA21. Other \( bm_3 \) inbreds equalled inbred MA21; inbred Tva-3904/2 \( bm_3 \), with a value of 21.47 MPa was least in the tested set.

At full maturity, puncture strength of stalk increased and ranged from 31.96 to 53.54 MPa. Inbreds Tva-3902 \( bm_3 \), Tva-3903 \( bm_3 \), Tva-3907/1 \( bm_3 \) and Tva-3907/2 \( bm_3 \) kept the statistical difference from the control, as did the inbred Tva-3902/2 \( bm_3 \). The stalk strength of these inbred lines exceeded 40 MPa. The rest of the inbreds did not differ statistically in stalk strength from the control MA21. At the milky-dough stage of maturity, some inbred lines had higher puncture strength than at full maturity. This fact is explained by the higher elasticity of stalk of these lines.

Table 1. Mean puncture strength (MPa) of stalks of the best \( bm_3 \) inbred lines at different stages of maturity. The control was MA21. \( n=3 \).

<table>
<thead>
<tr>
<th>Inbred line ( bm_3 )</th>
<th>Milky-dough maturity</th>
<th>Full maturity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tva-3901/1</td>
<td>46.78 ± 2.16</td>
<td>37.85 ± 2.64</td>
</tr>
<tr>
<td>Tva-3902/2</td>
<td>35.73 ± 2.05</td>
<td>44.81 ± 2.35</td>
</tr>
<tr>
<td>Tva-3902</td>
<td>50.21 ± 2.94</td>
<td>47.17 ± 2.15</td>
</tr>
<tr>
<td>Tva-3903</td>
<td>42.75 ± 4.31</td>
<td>41.77 ± 3.43</td>
</tr>
<tr>
<td>Tva-3904/1</td>
<td>36.08 ± 1.86</td>
<td>39.03 ± 1.17</td>
</tr>
<tr>
<td>Tva-3904/2</td>
<td>21.47 ± 3.33</td>
<td>30.98 ± 2.54</td>
</tr>
<tr>
<td>Tva-3904/3</td>
<td>34.42 ± 2.54</td>
<td>38.54 ± 1.76</td>
</tr>
<tr>
<td>Tva-3905</td>
<td>33.63 ± 1.47</td>
<td>39.61 ± 1.76</td>
</tr>
<tr>
<td>Tva-3906</td>
<td>35.20 ± 1.96</td>
<td>38.93 ± 1.17</td>
</tr>
<tr>
<td>Tva-3907/1</td>
<td>42.26 ± 1.76</td>
<td>46.77 ± 3.92</td>
</tr>
<tr>
<td>Tva-3907/2</td>
<td>46.48 ± 2.74</td>
<td>53.54 ± 4.60</td>
</tr>
<tr>
<td>Tva-3908/3</td>
<td>31.18 ± 2.64</td>
<td>34.12 ± 1.96</td>
</tr>
<tr>
<td>Tva-3908/2</td>
<td>31.38 ± 0.68</td>
<td>32.26 ± 2.45</td>
</tr>
<tr>
<td>Tva-3908/1</td>
<td>32.65 ± 1.27</td>
<td>31.96 ± 2.54</td>
</tr>
<tr>
<td>MA 21</td>
<td>34.51 ± 1.76</td>
<td>35.20 ± 1.47</td>
</tr>
<tr>
<td>LSD(_{0.05})</td>
<td>5.638</td>
<td>5.011</td>
</tr>
<tr>
<td>LSD(_{0.01})</td>
<td>7.806</td>
<td>6.943</td>
</tr>
</tbody>
</table>
Table 2. Puncture strength (MPa) of stalks of bm3 inbreds lines and normal control at different stages of maturity.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Milky-dough maturity</th>
<th>Full maturity</th>
</tr>
</thead>
<tbody>
<tr>
<td>bm3</td>
<td>36.77 ± 0.88</td>
<td>39.32 ± 0.78</td>
</tr>
<tr>
<td>normal</td>
<td>34.51 ± 1.76</td>
<td>35.20 ± 1.47</td>
</tr>
</tbody>
</table>

Table 3. Lignin content (g/kg) in fresh matter and puncture strength (MPa) of stalks of bm3 hybrids and normal control at different stages of maturity.

<table>
<thead>
<tr>
<th>Hybrid bm3</th>
<th>Lignin content</th>
<th>Milky-dough maturity</th>
<th>Full maturity</th>
</tr>
</thead>
<tbody>
<tr>
<td>TA 322</td>
<td>77.2</td>
<td>57.76 ± 1.47</td>
<td>51.28 ± 3.53</td>
</tr>
<tr>
<td>TA 323</td>
<td>75.9</td>
<td>53.05 ± 3.04</td>
<td>55.89 ± 3.04</td>
</tr>
<tr>
<td>TA 324</td>
<td>83.0</td>
<td>63.74 ± 2.94</td>
<td>56.68 ± 2.15</td>
</tr>
<tr>
<td>Control</td>
<td>97.8</td>
<td>49.91 ± 3.62</td>
<td>51.87 ± 3.33</td>
</tr>
</tbody>
</table>

1. Significantly different from control at probability 0.05.

From the general mutant evaluation among inbreds (Table 2), we conclude that there are no differences in stalk strength between mutant lines bm3 and controls, either at milky-dough or at full maturity. Both types tended to increase stalk strength at full maturity.

The stalk strength of bm2 mutants was tested also in three experimental hybrids (Table 3). At milky-dough maturity hybrids bm3 reached higher stalk strength than the control hybrid. Hybrid TA324 bm3 differed from the control, with a value of 63.74 MPa. There were no differences between bm3 hybrids and the control at full maturity. Hybrids TA322 bm3 and TA324 bm3 had lower stalk strength at full maturity than at milky-dough maturity. Lignin content in experimental hybrids was 77.61-84.87% of that of non-mutant controls. Between lignin content of bm3 hybrids and stalk strength, there was a certain relation that was not examined in detail.

Results of our study are considered preliminary. To explain the increased resistance to lodging of bm3 genotypes, a more detailed study of morphological and anatomical traits of stalk, e.g. those studied by Chang et al. (1976) and Foley & Clark (1984), is necessary. The method of measurement of puncture strength for penetration into stalk is not precise, because the final value is influenced also by stalk elasticity, as shown in values for some bm3 genotypes at milky-dough and full maturity.

Conclusions

1. One can increase resistance to stalk lodging in bm3 genotypes by appropriate breeding and by constant evaluation of stalk quality.
2. Measurement of puncture strength is useful for evaluation of general maize stalk quality.
References


Forage quality of high-protein opaque-2 maize hybrids of various complexity in relation to site and rate of fertilizer

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Maize Department, All-Union Institute of Plant Breeding and Genetics, Ovidiopolskaja Doroga 3, Odessa 36, USSR

Summary

The various types of high-protein hybrids of opaque-2 maize were developed on comparable genetic basis. There were single crosses, three-way crosses, backcrosses, double and complex (six-way) crosses. The trials examined effect of year, site, hybrid genotype and rate of fertilizer with N 280, P 120 and K 120 kg/ha on content of protein and lysine in grain and the whole plant, on ecological adaptability of different hybrid types. In the southern zone, single hybrids had highest contents of protein and lysine in grain and yield per area unit, and complex hybrids the lowest. In the northern zone, there were no significant differences between hybrids types in contents of protein and lysine. The main factorial component of variance and the highest contribution to phenotypic variability of lysine and protein in grain was the place of cultivation. The greatest response of protein and lysine in grain to favourable sites was in single hybrids, and the least in complex hybrids. The complex hybrids had much more ecological adaptability of these characters in comparison with other hybrid types. Protein and lysine increased significantly in leaf and stem and in cobs of high-protein and low-protein opaque-2 hybrids under high rates of fertilizer.

Descriptors: maize, Zea mays L., opaque-2, forage quality

Introduction

A large and unused resource for increasing of fodder quality of maize grain and forage is genetic improvement of content of protein and amino acids with the high-lysine mutant opaque-2. However it is not clear how this aim is linked up optimumly with genetic features of various hybrid maize types, which are distributed widely with present cultivation practice.

It is known that protein content of grain of single hybrids is higher with parents having a higher content (Frey, 1949; Kovarskiî & Pukalov, 1961; Kluchko & Maksak, 1969). The same trait is indicated for single hybrids with opaque-2 gene (Hadzhinov et al., 1976; Belousov & Mamatov, 1980). Single hybrids were developed from opaque-2 lines with grain protein content near 17-22%. The hybrids had 12-16% protein (Hadzhinov et al., 1976; Belousov & Mamatov, 1980). Protein content in single hybrid grain was usually lower than or intermediate between parents.

There is no agreement about comparative value of single and double hybrids for protein content. Some breeders consider that double hybrids have the same protein content as single ones (Gurzhiev, 1962; Kozubenko, 1965), but data of other researchers contradict that view (Pokrovskaja & Galeev, 1961).

1. Paper not presented at the meeting.
With the need for profitability of maize grain production, three-way hybrids have, however, gained ground in commercial growing, as well as single and double hybrids, and use of backcross hybrids is a topical question (Jugenheimer, 1979; Kljuchko & Mel’nik, 1977). Recently the idea of complex hybrids was conceived and approved (Kobelev & Kobeleva, 1984). Similar hybrid types can be used for breeding fodder maize with improved protein content and quality. No doubt, some of these hybrid types could be preferable in content of protein and essential amino acids in grain and forage, and in response to abiotic factors. Significant variability of protein content with environmental conditions of the site and year is well known. However, the effect of these factors has been studied for ordinary and high-lysine maize for only a few hybrid types, mainly single crosses. There has been no practical study on the influence of climatic factors on content and quality of protein in grain of different high-protein hybrids of opaque-2. This aspect of research would be helpful to explain some of the main features of protein heredity in various hybrid types and especially the contradictory data on protein content of single and double hybrids.

Nitrogen fertilizers are the main factor raising protein content of fodder maize. Their mode of action on protein content and quality of ordinary and high-lysine hybrids is known quite well (Frey, 1961; Pavlov, 1967; Zink & Wilberg, 1976; Maggiore, 1977; Efinfov & Naumenko, 1980). That is why it was interesting to study the effect of nitrogen fertilizers on protein and lysine content in forage of high-protein opaque-2 hybrids, especially for fuller estimation of their forage characteristics.

The aim of the paper was to develop the main types of maize hybrids with comparable genetic background from high-protein opaque-2 lines and to assess them for protein and lysine content in grain and forage in relation to environmental conditions and fertilizer treatment.

**Materials and methods**

Six high-protein opaque-2 lines were used as initial material to create hybrids (Table 1): single, three-way, backcross, double and complex (six-way) hybrids. Single hybrids were made by diallele scheme (Griffing, IV method, model I). In obtaining three-way, double and complex hybrids, the main aim was to ensure equal participation of all lines in each position of a certain hybrid type. Backcross hybrids were obtained by the scheme (A × B^2) × C (Kljuchko & Mel’nik, 1977), but an equal part of each line was provided only for positions B^2 and C to reduce the load of work. Six-way hybrids were obtained by scheme ((A × B) × C) × ((D × E) × F) (Kobelev & Kobeleva, 1984). On the whole, 15 single, 30 three-way, 24 backcross, 45 double and 60 complex hybrids were studied. Hybrid sets were sown as randomized blocks, in

**Table 1. Content of protein in forage and of lysine in protein of high-protein and lysine-rich opaque-2 lines.**

<table>
<thead>
<tr>
<th>Line</th>
<th>Content of protein (g/kg)</th>
<th>Content of lysine in protein (g/kg)</th>
<th>Mass of lysine per kernel (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Od 135 o2p</td>
<td>173</td>
<td>36.8</td>
<td>0.64</td>
</tr>
<tr>
<td>Go 100 o2p</td>
<td>187</td>
<td>37.9</td>
<td>0.71</td>
</tr>
<tr>
<td>Vir 44 o2p</td>
<td>145</td>
<td>41.8</td>
<td>0.61</td>
</tr>
<tr>
<td>D-6 o2p</td>
<td>182</td>
<td>40.0</td>
<td>0.73</td>
</tr>
<tr>
<td>1013 o2p</td>
<td>170</td>
<td>38.4</td>
<td>0.65</td>
</tr>
<tr>
<td>IHP BC o2p</td>
<td>202</td>
<td>38.0</td>
<td>0.77</td>
</tr>
</tbody>
</table>

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3 replicates in 1981-1982 at two sites: southern steppe (Zone D) and forest-steppe (Zone PK). Fertilizer was applied before ploughing at a rate of N 280, P 120 and K 120 kg/ha. Biochemical analysis were made in laboratory of plant biochemistry of the Institute under the supervision of Dr A.P. Levitski and senior research worker P.N. Pilneva.

Results and discussion

The hybrid types studied were significantly inferior to initial parent lines in protein content (Table 2) in both years and at both sites. Single hybrids had the highest protein content. They exceeded all other hybrid types in protein content of grain in the southern zone in each year. Backcross hybrids were an exception. In 1981, they were equal in protein content of grain to single crosses, but in 1982 they had significantly less protein than single hybrids. Complex hybrids accumulated much less grain protein on average than other hybrid types in southern zone.

Quite a different pattern was observed in the northern zone during the test years (Table 2). The protein content of grain in hybrids of all types was significantly less (by 15-30 g/kg than in the southern zone). These data are in a good accordance with variation in protein content of grain with the move from south to north. This variation has been assessed for many crops, including maize (Smirnova & Ikonnikova, 1958). It was established too that in the northern zone there was unexpectedly no significant difference between hybrid types in content of protein in grain. Three-way, backcross, double and complex hybrids had no significant differences from single hybrids in protein content. Such equalization of different hybrid types for protein content with the move to the north can explain the contradiction pointed out earlier, when double hybrids did not differ in protein content from single ones under the particular northerly conditions (Gurzhiev, 1962; Kozubenko, 1965), and in the same time they were inferior to single crosses under the more southerly conditions (Pokrovskaja & Galeev, 1961).

The broadest variation and combinations with the highest protein content were found for single crosses (206 g/kg) and backcrosses (197 g/kg). The other types of hybrids had about the same contents of protein.

There was no clear relationship between the complexity of high-protein opaque-2 hybrids and lysine content in protein. The lysine content changed in line with the well known negative correlation with protein content.

Table 2. Content of protein in grain (g/kg) of high-protein lines and different hybrid types of opaque-2 maize. Least significant difference (at \( P = 0.05 \)) between group means of different zones was 2, that between group means of different years was 2 g/kg.

<table>
<thead>
<tr>
<th>Type</th>
<th>Site D</th>
<th>Site PK</th>
<th>( \bar{x} )</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lines</td>
<td>194</td>
<td>168</td>
<td>155</td>
<td>125</td>
</tr>
<tr>
<td>Hybrids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>single</td>
<td>166</td>
<td>138</td>
<td>122</td>
<td>104</td>
</tr>
<tr>
<td>three-way</td>
<td>154</td>
<td>132</td>
<td>122</td>
<td>106</td>
</tr>
<tr>
<td>backcross</td>
<td>166</td>
<td>127</td>
<td>122</td>
<td>105</td>
</tr>
<tr>
<td>double</td>
<td>156</td>
<td>129</td>
<td>123</td>
<td>105</td>
</tr>
<tr>
<td>complex</td>
<td>145</td>
<td>127</td>
<td>121</td>
<td>109</td>
</tr>
<tr>
<td>LSD(_{0.05})</td>
<td>9</td>
<td>6</td>
<td>4</td>
<td>3</td>
</tr>
</tbody>
</table>
Lysine content of grain, like protein content, had some relationship with hybrid type and site. Single crosses had a significant advantage in this trait over double and particularly over complex hybrids at the southern site: in the north, these hybrids showed practically no differences (Table 3).

The most important integral indicator of acceptability of high-protein and lysine-rich maize hybrids is yield of protein and lysine. For protein yield, results conformed to expectations from the protein and lysine content in grain: at the southern site, single hybrids gave significantly higher yield of protein than three-way, double and complex hybrids (Table 4). Complex hybrids gave the lowest protein yield in average. Backcross hybrids were inferior to single crosses too, but in 1981 the differences were not significant. At the northern site (PK), all hybrid types had no significant differences in protein yield (Table 4).

The advantage of single crosses also existed for lysine yield (Table 5). Some three-way crosses and backcrosses were close to them. Complex and double hybrids had the lowest lysine yield. These hybrids, especially the complex ones, had significantly smaller range and smaller maximum yields of protein and lysine (Tables 4 and 5). These data and the significantly different range in protein of various hybrid types (Table 2) show the limited opportunities of selection of complex and double hybrids for maximum content and yield of protein and lysine. However the result can be quite different with larger sets of initial lines and by application of special methods for selection of parents (Kobelev & Kobeleva, 1984).

Table 3. Lysine content (g/kg) in protein of grain of high-protein lines and different hybrid types of opaque-2 maize. Least significant difference \((P = 0.05)\) between group means of different zones was 0.2; that between group means of different years 0.1.

<table>
<thead>
<tr>
<th>Type</th>
<th>Site D</th>
<th></th>
<th>Site PK</th>
<th></th>
<th>(\bar{x})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lines</td>
<td>0.65</td>
<td>0.61</td>
<td>0.58</td>
<td>0.48</td>
<td>0.58</td>
</tr>
<tr>
<td>Hybrids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>single</td>
<td>5.3</td>
<td>5.2</td>
<td>4.5</td>
<td>3.9</td>
<td>4.7</td>
</tr>
<tr>
<td>three-way</td>
<td>5.0</td>
<td>5.2</td>
<td>4.3</td>
<td>4.0</td>
<td>4.6</td>
</tr>
<tr>
<td>backcross</td>
<td>5.3</td>
<td>4.6</td>
<td>4.4</td>
<td>3.9</td>
<td>4.6</td>
</tr>
<tr>
<td>double</td>
<td>4.9</td>
<td>4.7</td>
<td>4.4</td>
<td>3.8</td>
<td>4.5</td>
</tr>
<tr>
<td>complex</td>
<td>4.8</td>
<td>4.6</td>
<td>4.5</td>
<td>4.1</td>
<td>4.5</td>
</tr>
<tr>
<td>LSD(_{0.05})</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>(0.2)</td>
</tr>
</tbody>
</table>

Table 4. Protein yield (kg/ha) of high protein opaque-2 maize hybrids of different types. Least significant difference \((P = 0.05)\) between group means of different zones was 43.5; that between group means of different years was 46.4.

<table>
<thead>
<tr>
<th>Type</th>
<th>Site D</th>
<th></th>
<th>Site PK</th>
<th></th>
<th>(\bar{x})</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single</td>
<td>703.6</td>
<td>665.5</td>
<td>517.2</td>
<td>509.7</td>
<td>599.0</td>
<td>368-931</td>
</tr>
<tr>
<td>Three-way</td>
<td>616.6</td>
<td>626.1</td>
<td>498.1</td>
<td>502.9</td>
<td>560.9</td>
<td>378-860</td>
</tr>
<tr>
<td>Backcross</td>
<td>694.0</td>
<td>593.1</td>
<td>476.9</td>
<td>509.0</td>
<td>568.3</td>
<td>375-919</td>
</tr>
<tr>
<td>Double</td>
<td>651.7</td>
<td>614.6</td>
<td>486.0</td>
<td>495.5</td>
<td>562.0</td>
<td>404-790</td>
</tr>
<tr>
<td>Complex</td>
<td>565.9</td>
<td>619.4</td>
<td>464.8</td>
<td>512.6</td>
<td>540.7</td>
<td>447-731</td>
</tr>
<tr>
<td>LSD(_{0.05})</td>
<td>46.4</td>
<td>35.3</td>
<td>20.0</td>
<td>19.5</td>
<td>19.3</td>
<td>(0.05)</td>
</tr>
</tbody>
</table>
Table 5. Lysine yield (kg/ha) of high-protein opaque-2 maize hybrids of different types. Least significant difference ($P = 0.05$) between group means of different zones was 16.5; that between group means of different years was 12.4.

<table>
<thead>
<tr>
<th>Type</th>
<th>Site D 1981</th>
<th>Site D 1982</th>
<th>Site PK 1981</th>
<th>Site PK 1982</th>
<th>$\bar{x}$</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single</td>
<td>22.5</td>
<td>24.9</td>
<td>18.9</td>
<td>19.0</td>
<td>21.3</td>
<td>13.2-33.4</td>
</tr>
<tr>
<td>Three-way</td>
<td>20.0</td>
<td>24.7</td>
<td>17.4</td>
<td>19.8</td>
<td>20.5</td>
<td>13.6-31.0</td>
</tr>
<tr>
<td>Backcross</td>
<td>22.2</td>
<td>21.4</td>
<td>17.4</td>
<td>18.9</td>
<td>20.0</td>
<td>13.5-30.7</td>
</tr>
<tr>
<td>Double</td>
<td>20.5</td>
<td>22.4</td>
<td>17.6</td>
<td>18.1</td>
<td>19.7</td>
<td>14.2-27.8</td>
</tr>
<tr>
<td>Complex</td>
<td>19.0</td>
<td>22.4</td>
<td>16.8</td>
<td>18.5</td>
<td>19.2</td>
<td>16.1-26.3</td>
</tr>
<tr>
<td>LSD$_{05}$</td>
<td>1.2</td>
<td>1.0</td>
<td>1.0</td>
<td>0.8</td>
<td>0.7</td>
<td></td>
</tr>
</tbody>
</table>

So, the main quality traits of complex and double hybrids, such as protein and lysine content, and the quantitative traits protein and lysine yield were significantly lower than of single crosses in the southern zone. Backcross and three-way hybrids had the same values as particular single crosses. However in the northern zone the various high-protein hybrid types showed practically no differences.

For effective utilization of maize hybrids with increased protein content and improved quality the role of environment, particularly site and year, on variation in major traits of grain quality are very important. A significant effect has been found of these factors on protein content of ordinary maize (Smirnova-Ikonnona, 1958; Éke et al., 1984). We studied effects of seasonal conditions (Y), site (L) and genotype (G) on protein and lysine content of high-protein opaque-2 hybrids. These factors were studied as random effects and analysed as a trifactorial dispersion complex. The components of variance of simple and three-way interactions were determined by Snedecor's method (Snedecor, 1961), and effects of major factors by the methods of Cochran (1951) and Satterthwaite (1946).

Analysis of variance and its factorial components demonstrated (Table 6), that only years and G × Y × L interaction did not significantly affect variation in grain protein. The effect of all other factors was highly significant ($P = 0.01$), except for the interaction G × Y ($P = 0.05$). Site had the greatest effect on hybrid phenotypic variability of content of grain protein (the

Table 6. Analysis of variance and components of variance of high-protein opaque-2 hybrids for protein and lysine contents of grain. df, degrees of freedom; ms, mean-square; *, ** significant at $P = 0.05$ and $P = 0.01$, respectively.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Protein</th>
<th>Lysine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df ms</td>
<td>component of variance</td>
</tr>
<tr>
<td>Year</td>
<td>1 62.220 1.998</td>
<td>1 0.0088 0.0002</td>
</tr>
<tr>
<td>Site</td>
<td>1 140.700** 4.616</td>
<td>1 0.0713 0.0027</td>
</tr>
<tr>
<td>Genotype</td>
<td>4 29.323** 2.394</td>
<td>3 0.0018 -0.0015</td>
</tr>
<tr>
<td>Year × site</td>
<td>1 1.980* 0.129</td>
<td>1 0.0043* 0.0003</td>
</tr>
<tr>
<td>Genotype × year</td>
<td>4 0.354* 0.051</td>
<td>3 0.0009 0.0001</td>
</tr>
<tr>
<td>Genotype × site</td>
<td>4 1.535 0.496</td>
<td>3 0.0026* 0.0004</td>
</tr>
<tr>
<td>Genotype × year × site</td>
<td>4 0.048 -0.018</td>
<td>3 0.0002 0.0000</td>
</tr>
<tr>
<td>Error</td>
<td>32 0.101 0.101</td>
<td>32 0.0002 0.0002</td>
</tr>
</tbody>
</table>
factorial component of variance 4.616; fraction of total variance 0.592) and so genotype had component of variance 2.394; fraction 0.307). However the magnitude of the component of variance for site was about twice as high as the genotypic component of variance.

Only site had a highly significant effect on lysine content of grain (P = 0.01), effects of the Y \times L and G \times L interactions were significant at P = 0.05 (Table 6). The influence of other effects and their interactions was not significant. The factorial component for L was significantly higher than all others, suggesting the predominant contribution of site for content of lysine in hybrid grain.

Thus the strong and predominant effect of site over other tested factors for content of protein and lysine has been determined in grain of high-protein opaque-2 hybrids.

Comparative analysis of the effect of environment on grain quality of each tested hybrid type separately is of great interest. The stability of parameters of grain quality has important practical significance for choice of the most acceptable hybrid type for the specific farming conditions.

Significant differences in variability and ecological adaptability between hybrid types in productivity and its components have been demonstrated on ordinary maize (Kaminskaja et al., 1971; Eberhart & Russell, 1966; 1969; Weatherspoon, 1970). We have studied ecological adaptability of various high-protein opaque-2 hybrids for protein and lysine content and lysine yield. For calculation of stability parameters, the regression model of Eberhart & Russell (1966; 1969) was used, and coefficients of variation were estimated as well. Years (1982, 1983) and sites (D, PK) were used as variable factors.

Comparison of regression coefficients for all three traits showed strong differences between the hybrid types in response to conditions (Table 7). The highest variation in content of protein (P) and lysine (L) and in lysine yield (LY) with environment conditions was observed in single hybrids ($b_P = 1.28; b_L = 1.34; b_{LY} = 1.21$). These hybrids had the maximum parameters of yield quality under favourable conditions.

<table>
<thead>
<tr>
<th>Hybrid type</th>
<th>$\bar{x} \pm s_x$</th>
<th>$V$</th>
<th>Regression of coefficient $b \pm s_b$</th>
<th>Variance of stability $s^2_y$</th>
<th>Ranking for environmental response</th>
<th>Ranking for stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein content of grain (g/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>single</td>
<td>133 ± 6.9</td>
<td>180</td>
<td>1.28 ± 0.052</td>
<td>61.7</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>three-way</td>
<td>129 ± 5.3</td>
<td>140</td>
<td>0.98 ± 0.027</td>
<td>36.2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>double</td>
<td>128 ± 5.5</td>
<td>150</td>
<td>1.02 ± 0.030</td>
<td>39.4</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>complex</td>
<td>126 ± 3.9</td>
<td>100</td>
<td>0.72 ± 0.041</td>
<td>19.9</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Lysine content (g/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>single</td>
<td>4.7 ± 0.33</td>
<td>140</td>
<td>1.34 ± 0.024</td>
<td>0.064</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>three-way</td>
<td>4.6 ± 0.28</td>
<td>120</td>
<td>1.12 ± 0.227</td>
<td>0.045</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>double</td>
<td>4.5 ± 0.24</td>
<td>110</td>
<td>0.95 ± 0.180</td>
<td>0.032</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>complex</td>
<td>4.5 ± 0.15</td>
<td>70</td>
<td>0.59 ± 0.093</td>
<td>0.013</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Lysine yield (kg/ha)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>single</td>
<td>21.3 ± 0.88</td>
<td>0.12</td>
<td>1.21 ± 0.150</td>
<td>7.23</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>three-way</td>
<td>20.5 ± 0.82</td>
<td>0.11</td>
<td>1.09 ± 0.151</td>
<td>6.03</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>double</td>
<td>19.7 ± 0.65</td>
<td>0.10</td>
<td>0.88 ± 0.109</td>
<td>3.94</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>complex</td>
<td>19.2 ± 0.61</td>
<td>0.09</td>
<td>0.82 ± 0.110</td>
<td>3.41</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>
The complex hybrids had the least response to conditions improving protein and lysine storage in grain \((b_P = 0.72, b_L = 0.59, b_{LY} = 0.82)\). Three-way and double hybrids were intermediate, but three-way hybrids were often close to single crosses and double hybrids to complex hybrids (Table 7).

Thus the size of the response of hybrids to environmental conditions and their ability to use favourable conditions effectively to accumulate protein and lysine in grain show a clear decrease from single to complex hybrids.

Results of comparison of different hybrid types for stability were quite conflicting (Table 7). The least stability variance \((s^2_d)\), and the highest ecological adaptability of all three traits were found in the complex hybrids \((s^2_{dP} = 1.99; s^2_{dL} = 0.0013; s^2_{dLY} = 3.41)\). Single hybrids had the lowest stability, showing the greatest variances \((s^2_{dP} = 6.17; s^2_{dL} = 0.0064; s^2_{dLY} = 7.23)\). Three-way and double hybrids occupied intermediate ranges, but stability of double hybrids for lysine content of grain and lysine yield was significantly higher than of three-way hybrids (Table 7). The results of regression analysis for lysine content of grain (Fig. 1) demonstrated the response of each hybrid type to environmental changes. Single hybrids had the steepest regression line but under unfavourable conditions single crosses, three-way crosses and double hybrids can contain even less protein and lysine in grain than complex ones. Comparable results were obtained for protein content and yield. So complex hybrids can achieve the same protein and lysine contents as single and three-way crosses under unfavourable conditions.

Significant differences between hybrids in the nature and strength of response to environmental conditions can be explained by various level of heterogeneity of F1 hybrid populations. Genotypic diversity and level of heterozygosity in F1 plants of complex hybrids are higher than in other types, and especially in comparison with single hybrids. Perhaps these characteristics

![Fig. 1. Regression lines of high-protein opaque-2 hybrids for lysine content of grain with changes in environmental conditions.](image-url)

<table>
<thead>
<tr>
<th>Hybrid Type</th>
<th>Slope (b)</th>
<th>Intercept (a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single crosses</td>
<td>4.7</td>
<td>13.4</td>
</tr>
<tr>
<td>Three-way</td>
<td>4.6</td>
<td>11.2</td>
</tr>
<tr>
<td>Double</td>
<td>4.5</td>
<td>9.5</td>
</tr>
<tr>
<td>Complex</td>
<td>4.5</td>
<td>5.9</td>
</tr>
</tbody>
</table>
are the basis of the higher homoeostasis and 'bufferness' of complex hybrids for the major
traits of yield quality. Similar results were obtained in productivity tests of single, three-way
and double hybrids of ordinary maize (Eberhart & Russell, 1969; Weatherspoon, 1970). Some
reports state that some single crosses were as stable in yield as double hybrids (Eberhart &
Russell, 1969; Hadzhinov, 1973. There is a possibility that extensive ecological testing of high-
lysine hybrids may result in selecting hybrids with high stability of the main parameters of
grain quality.

Fertilizer rate, especially nitrogen, is a very important environment factor. Its effect on
forage quality of various high-protein opaque-2 hybrid types has not yet been studied.

For two years, we have been studying the effect of high nitrogen rate (280 kg/ha) in combi-
nation with P (120 kg/ha) and K (120 kg/ha) on protein and lysine content of forage and
different morphological parts of single, three-way and double high-protein opaque-2 hybrids.
High-lysine single cross Krasnodarsky 303 H1 with normal content of protein was used as
control. High-protein opaque-2 hybrids averaged significantly more than the unfertilized con-
trol for protein content in stover (by 72%), stalks (by 18%) and ears (by 17%). And what is
more, they significantly exceeded (by 15-25%) the low-protein control on protein content of
all plant parts either with or without fertilizers (Table 8).

With fertilizers, protein quality was slightly lower, but lysine content in dry-matter of stover
and ears increased significantly (by 61 and 10%, respectively). The high-lysine hybrid with
normal content of protein had similar response to fertilizer, but this hybrid was significantly
inferior to high-protein hybrids for lysine content in dry-matter (by 18-39%) although its
protein quality was slightly higher. Single and three-way hybrids significantly exceeded double

Table 8. Protein content (g/kg) in morphological parts of high-protein and low-protein opaque-2 hybrids

<table>
<thead>
<tr>
<th>Hybrid type</th>
<th>Stover control</th>
<th>Stover dressed</th>
<th>Stalks control</th>
<th>Stalks dressed</th>
<th>Ears control</th>
<th>Ears dressed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single</td>
<td>54</td>
<td>86</td>
<td>82</td>
<td>90</td>
<td>97</td>
<td>104</td>
</tr>
<tr>
<td>Three-way</td>
<td>53</td>
<td>92</td>
<td>67</td>
<td>80</td>
<td>91</td>
<td>109</td>
</tr>
<tr>
<td>Double</td>
<td>46</td>
<td>86</td>
<td>56</td>
<td>69</td>
<td>76</td>
<td>95</td>
</tr>
<tr>
<td>Mean</td>
<td>51</td>
<td>88</td>
<td>68</td>
<td>80</td>
<td>88</td>
<td>103</td>
</tr>
<tr>
<td>Krasnodarsky 303 H1</td>
<td>41</td>
<td>76</td>
<td>55</td>
<td>66</td>
<td>71</td>
<td>82</td>
</tr>
<tr>
<td>LSDₜ₀</td>
<td>6</td>
<td>9</td>
<td>2</td>
<td>3</td>
<td>8</td>
<td>9</td>
</tr>
</tbody>
</table>

Table 9. Lysine content (g/kg) of morphological parts of high-protein and low-protein opaque-2 maize
hybrids dressed with fertilizer (average for 1980-1981 N 280, P 120 and K 120 kg/ha).

<table>
<thead>
<tr>
<th>Hybrid type</th>
<th>Stover control</th>
<th>Stover dressed</th>
<th>Ears control</th>
<th>Ears dressed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single</td>
<td>1.9</td>
<td>2.8</td>
<td>3.2</td>
<td>3.4</td>
</tr>
<tr>
<td>Three-way</td>
<td>1.8</td>
<td>3.0</td>
<td>3.0</td>
<td>3.3</td>
</tr>
<tr>
<td>Double</td>
<td>1.6</td>
<td>2.8</td>
<td>2.5</td>
<td>2.9</td>
</tr>
<tr>
<td>Mean</td>
<td>1.8</td>
<td>2.9</td>
<td>2.9</td>
<td>3.2</td>
</tr>
<tr>
<td>Krasnodarsky 303 H1</td>
<td>1.4</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>LSDₜ₀</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>
hybrids in protein and lysine content in stover and in ears with or without fertilizer.

Our data demonstrate a significant increase in feeding quality of forage from opaque-2 hybrids with high and ordinary protein content as a result of high rate of fertilizers, in particular nitrogen. This increase was associated with higher content of protein and lysine in dry-matter of forage. High-protein opaque-2 hybrids considerably exceeded low protein opaque-2 hybrids for protein and lysine content in forage on fertilized and unfertilized plots.

References

Belousov, A.A. & N.A. Mamatov, 1980. Diallel'nyj analiz kombinacionnoj sposobnosti vysokobelkovo-vysokolizinovoj linij kukuruzy opejk-2 po urozhaju zerna i soderzhaniju v nem proteina. (Diallelic analysis of combining ability of high-protein and high-lysine lines of maize opaque-2 for yield of grain and content of protein in grain.) Selekcija, semenovodstvo i agrotehnika kukuruzy na juge Ukrainy. (Breeding, seed production and cropping methods for maize in southern Ukraine.) Odessa. p.32-40.


Selection criteria for silage maize
Ideal type of forage maize hybrid (*Zea mays* L.)

Lajos Pinter

Forage Research Institute, Iregszemcse, H-7095 Hungary

Summary

Special silage maize can produce high yields with high energy content and can be eaten by ruminants in large amounts. This statement involves some parameters which were analysed in focusing on the question whether the best grain hybrid equals the best forage one.

For yield of dry matter, a close relationship does not exist between forage and grain hybrids. In a continental climate, the ideal silage maize hybrid requires a proportion of grain of at least 30%. It is better to use the grain hybrid for a high-cutter harvesting system. Because standability cannot be characterized only by stalk strength and root lodging, we have to consider also leaf area. That is why silage hybrids must have an appropriate stalk strength and good resistance to root lodging too. The non-structural carbohydrate content of stover is important for high quality. It could be increased by using a male-sterile hybrid mixed with fertile one. The protein content, digestibility and intake of stover differ between the ideal grain and silage hybrids. The forage hybrid produces more assimilates than the grain can store. The surplus as non-structural carbohydrate is stored mainly in the stalk below the ear.

Descriptors: forage maize, *Zea mays* L., ideal type, dry matter yield, harvest index, standing ability, carbohydrate content, protein content, digestibility, dry matter intake

Hungary is one of the top countries in the world for grain-maize production. The same cannot be said of silage-maize production, although our climate is more suitable for the latter. According to a careful estimate, silage maize covers about 30% of the total maize area in Hungary. That is why the following question often arises.

What are the criteria for forage maize?

This has been discussed at several Eucarpia Congresses, first by Gunn (1975). A correct answer for every country and cropping system is difficult to give, because for choice of forage hybrid we have to pay attention not only to yield as for a grain hybrid, but also to many quality features. These depend on climate and on growing systems.

According to investigations of Hunter et al. (1977) and Hunter (1980), the longer photophase (20 vs 10 h) and cooler temperature (20 vs 30 °C) produced a higher mass of dry matter for whole plant. At the same time, they found significant differences in response to photophase and temperature by different genotypes.

The quality features of stover, lignin content and digestibility in vitro were better under low light intensity (Deinum & Bakker, 1981; Struik & Deinum, 1982) and cooler temperature (Adelana & Milbourn, 1972; Deinum & Struik, 1982). The quality of whole plant was signifi-
cantly influenced also by N fertilizer and cattle slurry (Dubas, 1974; Pain et al., 1977). Our results (Table 1) confirm statements of Fairey (1982). With increasing plant density, the quality features (e.g. protein content) decrease.

With such facts, we can outline only generalities about the ideal forage maize hybrid. The ideal silage maize can produce high yields with a high energy content, and can be eaten by ruminants in large amounts. This general definition involves some parameters about them. The following question often arises.

**Does the best grain hybrid equal the best forage one?**

**Dry matter yield** Maize breeders accepted choice of forage hybrids according to their grain-yielding ability. This approach is based on research in the United States in the 1930s and 1940s (Neves, 1933; Neves & Dungan, 1942). According to these early workers, the best grain genotypes were the best forage ones.

Gallais & Pollacek (1975) and Fairey (1980) concluded that the best grain hybrid did not equal the best forage one. Confirming this statement, Vattikonda & Hunter (1983) investigated many hybrids for grain and silage performance at two sites in Ontario. Correlations of only $r^2 0.23$ and 0.25 were observed. They concluded that separate grain and forage performance trials should be run to recommend for proper production.

**Harvest index** The findings of researchers working under different climatic conditions differ about ideal harvest index. Perry & Caldwell (1969), and Bunting (1976) found that there was no need for grain, because assimilates were stored in the vegetative parts in a similarly well digestible form as in the kernels. Smith et al. (1963), and Fischer et al. (1968) emphasize the importance of a large proportion of kernel. The differences of opinions can be attributed to differences in light intensity and temperature.

An investigation was conducted to determine the ideal harvest index in Hungary. Isolating ear primordia, different harvest index treatments were obtained at same plant density with the same genotype. After ensiling different treatments, digestibility was measured with sheep to calculate content of net energy. We established that the ideal silage hybrid needs a grain fraction of at least 300 g/kg (Figure 1). If cutting height was increased, the energy concentration could be raised according to earlier publications (Fisher et al., 1968; Gunnis & Burns, 1969; Andrieu & Demarquilly, 1974). Attention was not focused on whether the grain or the forage hybrid was more suitable for this purpose.

Using a special grain and a silage hybrid, a field trial was carried out with two cutting treatments in Hungary. If cut below the ear, the harvest index was better than in whole plant for both hybrids (Table 2). Conform the finding of Gross (1981), we have to harvest maize earlier in the high than in the low cutting treatment. According to DM yield results if we would like to produce whole plant silage we have to sow silage hybrid, but in case of concentrated silage it is better to use grain one.

| Table 1. Effect of plant density on protein content in dry matter of stover. Least significant difference ($P = 0.05$) was 5.1 g/kg. |
|---------------------|---|---|---|---|---|---|
| Plant density (m$^2$) | 4 | 6 | 8 | 10 | 12 | 14 |
| Protein content (g/kg) | 64.6 | 53.1 | 46.3 | 43.7 | 45.8 | 45.1 |

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Fig. 1. Relation of harvest index to content of net energy. ***, \( P = 0.01 \). Harvest index is mass ratio of grain to stover.

Table 2. Effect of cutting height on agronomic traits. Harvest index is mass ratio of grain to stover.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hybrid</th>
<th>Cutting height</th>
<th>LSD 5%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>at soil surface</td>
<td>below the ear</td>
</tr>
<tr>
<td>Harvest index (%)</td>
<td>forage</td>
<td>35.1</td>
<td>39.9</td>
</tr>
<tr>
<td></td>
<td>grain</td>
<td>42.5</td>
<td>53.0</td>
</tr>
<tr>
<td>Content of dry matter (g/kg)</td>
<td>forage</td>
<td>28.6</td>
<td>35.7</td>
</tr>
<tr>
<td></td>
<td>grain</td>
<td>30.1</td>
<td>37.4</td>
</tr>
<tr>
<td>Yield of dry matter (kg/m²)</td>
<td>forage</td>
<td>1.29</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>grain</td>
<td>1.12</td>
<td>1.02</td>
</tr>
</tbody>
</table>

**Standability**  It is well known that standability depends on the stalk strength, root lodging and the leaf area exposed to wind. Root lodging and leaf area depend mainly on the genotype. Stalk strength is determined by the rind strength and health of the pith tissue. As the pith is generally healthy at silage harvest, the rind strength is primarily responsible for stalk stability.

Rind strength shows a positive correlation with the lignin content (Gunn, 1975; Twumasi-Afriyie & Hunter 1982). In practice, ruminants cannot digest lignin. They concluded that high stalk strength was not desirable for a silage hybrid. However, in a Hungarian field trial, a significant decrease in yield of dry matter was found because of weak stalks damaged by a gale before flowering (Pinter et al., 1981).

Standability, characterized by rind puncture and by leaf area, was investigated for four hybrids in Hungary. The most critical stage was tasselling because development of stalk strength was then not so intense as of leaf area (Figure 2). Conform the earlier statement, I found a close correlation between stalk strength and lignin content (Table 3). As standability...
is influenced not only by the stalk strength, but also by leaf area, the two parameters should be analysed together. Thus I examined the correlation between reaching a force of 1.0 N for rind puncture and lignin content and found, instead of the previous close correlation, an insignificant one \( r = 0.17 \). So we have to reconsider the earlier statement. Also for a forage hybrid, we need an appropriate rind resistance.

**Carbohydrate content** Forage quality is influenced by non-structural and structural carbohydrates. The non-structural forms are sugar and starch. The structural form that most influences forage quality is lignin.

Before we attempt to discuss this subject, let me recall the idea of sink and source, which was summarized and elaborated by Tollenaar (1977) for grain maize. For forage maize, the sink means not only the grain but all aerial parts, because grain and vegetative parts are used.

Many researchers have dealt with brown-midrib mutants, which contain a small amount of lignin and which store a substantial proportion of assimilates as carbohydrate in the stalk; consequently its digestibility is better than normal forage maize (Kuc & Nelson, 1964; Muller et al., 1971; Colenbrander et al., 1973; Hartley & Jones, 1978; Gallais et al., 1980). Investigating 130 normal and 130 bm\textsuperscript{3} genotypes, Miller & Geadelmann (1983) concluded that early
vigour, duration of vegetative development and grain-filling period of the latter were noted respectively for 10, 13 and 15% less than among normal genotypes. Yields of grain, stover and feed of bm
3 were 23, 10 and 16% less than among normal genotypes (Miller et al., 1983). Except for stover yield, no bm
3 genotype performed better than normal genotypes. In a feeding trial, grain added to the ration to 2.0% of body weight caused the benefit of bm
3 to disappear (Keith et al., 1981).

Barren plants have been produced by the use of male-sterile cytoplasm, by early removal of ear shoots or by enclosing the ear shoot in bags before emergence of silks. Assimilates cannot be accumulated to the grain, so the sink is the vegetative plant parts, mainly stalks (Schmid et al., 1969; Cummins & McCullough, 1971; Marten & Westerberg, 1972; Iwata, 1973; Phipps, 1980). This stover contains more carbohydrate, mainly sugar, consequently it has better digestibility than that of fertile analogues. The yield of dry matter of cms hybrids was less than of non-barrens (Perry & Caldwell, 1969; King et al., 1982; Deinum & Knoppers, 1979), because of the inhibitory effect of the grain ‘sink’ on carbohydrate accumulation in the stover (Moss, 1962; Tollenaar & Daynard, 1982). Bunting (1975), summarizing the literature, suggested that yield of dry matter was much less dependent on grain formation in a source limiting than in a sink limiting area.

Pollen development uses a lot of energy, which is saved in cms genotypes. If pollination can be assured by mixing cms with fertile plants and the grain sink is large enough (e.g. two ears per plant) to accumulate the surplus, it can be exploited to increase grain yield (Grogan, 1965). If the grain ‘sink’ is limited, related to the surplus, surplus assimilates can be stored in the stover (M.E.V. Lourenço, Ames, Iowa, personal communication). That would be a tool to improve forage production.

Autotetraploid genotypes have larger stover than diploid ones, which could benefit forage production. Atlin & Hunter (1985), conducting a trial at a source-limiting area (in Ontario) did not show any benefit of autotetraploidy.

It is well known that black-layer formation is finished earlier in opaque hybrids than in normal ones. Consequently a substantial amount of surplus assimilates accumulates in the stover. They could be used in forage production.

Protein content Although ruminants can produce protein, the highest weight gain was obtained on a ration with 13.3% crude protein (Sambot et al., 1980). Since genotypes differ in protein content of whole plant (Gross, 1980; Maggiore et al., 1980; Mann & Pollmer, 1981) improvement is possible.

Schwab et al. (1980) investigated the protein content of different plant parts of some hybrids. The largest differences were observed in the stalk of hybrids.

Digestibility and dry matter intake Genotype differences have been demonstrated for intake of dry matter (Andrieu & Demarquilly, 1974; Gallais et al., 1976).

For eight whole plant silages with a grain ration of between 39.0 and 51.0%, intake and digestibility were not strongly linked to ear-to-stover ratio (Gallais, 1976).

Yield of digestible dry matter Grain and forage maize hybrids were separated into two maturity groups on the basis of yield of digestible dry matter in Hungary. In each group, forage hybrids yielded more than grain ones (Table 4). Contribution of forage hybrids to increasing yield were similar in the two maturity groups.

For grain hybrids, 7.5% more digestible dry matter was obtained from ear than for forage
Table 4. Comparison of grain and forage maize hybrids for relative yield of digestible dry matter in various plant parts (%).

<table>
<thead>
<tr>
<th>Plant part</th>
<th>Grain hybrids</th>
<th>Forage hybrids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early maturing group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>whole plant</td>
<td>100.0</td>
<td>128.8</td>
</tr>
<tr>
<td>ear</td>
<td>100.0</td>
<td>104.1</td>
</tr>
<tr>
<td>remainder stored in stover</td>
<td>11.8</td>
<td></td>
</tr>
<tr>
<td>Late maturing group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>whole plant</td>
<td>100.0</td>
<td>128.8</td>
</tr>
<tr>
<td>ear</td>
<td>100.0</td>
<td>115.4</td>
</tr>
<tr>
<td>remainder stored in stover</td>
<td>13.4</td>
<td></td>
</tr>
</tbody>
</table>

Table 5. Relative differences from grain hybrids in distribution of digestible dry matter for plant parts of forage hybrids.

<table>
<thead>
<tr>
<th>Plant part</th>
<th>Relative difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ear</td>
<td>+ 7.5</td>
</tr>
<tr>
<td>Stover below the ear</td>
<td>- 4.8</td>
</tr>
<tr>
<td>Stover above the ear</td>
<td>- 2.7</td>
</tr>
</tbody>
</table>

ones (Table 5). For forage hybrids the largest part, that is 64.0% of increased digestible dry matter was accumulated in stover below the ear and only 36.0% above the ear. This confirms that we have to consider whether it is worse to harvest the forage with a high cutter.

Finally let me stress that even for one place the ideal type of forage maize must be reconsidered continuously to take account of changes in demand.

References


Characterization of silage maize: Patterns of dry matter production, LAI evolution and feeding value in late and early genotypes

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Summary

Early and late maize varieties were compared by sampling plants 4 times after silking of early hybrids. At silking, yield of whole plant for early genotypes was the same as of later genotypes even if leaf area index of later ones was about twice as high. But after silking of the latest genotypes, the higher photosynthetic efficiency of physiologically younger leaves gave rise to a higher yield of whole plant for later genotypes, related to an apparently better leaf area duration. Better silage yields are not obtained from hybrids with a high proportion of grain. Feeding trials with sheep show that feeding values were about 0.10-0.15 UFL/kg less for later varieties than for earlier ones; but yields of net energy were about 1.5 times higher than for the latest genotype. If farmers are to optimize production of silage maize in relation to the performance level of their herd and the intake of silage desired, they need to base their choice of hybrid on net-energy content and on earliness.

Descriptors: silage maize, Zea mays L., leaf area index, brown mid-rib, feeding value

Introduction

A good variety of maize for silage must lead to the highest profit of the crop after conversion by cattle. But the qualities required of a silage maize hybrid differ according to the performance of the individual animal and with cost of energy-rich concentrates. So characteristics of a variety lie between those of high-yielding genotypes (about 20 t/ha), with quite a low content of dry matter (270 g/kg) and fairly low energy content, and genotypes with a high energy content, quite a high content of dry matter (about 350 g/kg), but low yield (about 10 t/ha). This study of patterns of dry-matter production and feeding value in early and late genotypes should contribute to the definition of a silage maize ideotype concerning whole-plant yield, earliness, and balance between yield and feeding value for cattle.

Material and methods

For small plot trials 10 commercial hybrids were used:

- Brulouis, Leader early FAO less than 250
- Baron, Dea medium-early FAO between 250 and 350
- Mohican, LG 22 medium-late FAO between 350 and 450
- Roc, Tador late FAO between 500 and 700
- Lorena, SNH 931 very late FAO more than 800

The hybrids were sown on 25 April 1984 at Lusignan (France; latitude 46.5°N; altitude
about 100 m) at a plant density of 80 000 ha\(^{-1}\). Irrigation was provided at 60 mm to prevent water stress (rainfall only 22, 47 and 66 mm during June, July and August, respectively, with an average monthly temperature of 16.5, 19.6 and 18.6 °C). Medium-early genotypes are well adapted as grain maize, and medium-late as silage maize for these local conditions. Of each hybrid, 6 plants were sampled 4 times in the growing season according to a randomized block design with 3 replicates:

1. about mid-silking of early and medium-early hybrids (1 August).
2. about mid-silking of medium-late hybrids (14 August).
3. 1 month after mid-silking of early hybrids (3 September).
4. 2 months after mid-silking of very late hybrids (26 October).

After the third sampling, plants were separated into stover (stalk and leaves) and ears (husks, cob and grains). Dry-matter content was estimated by oven-drying for 48 h at 85 °C. Leaf area was calculated by the classical formula length \(\times\) width \(\times\) 0.75, corrected by the proportion of green parts.

To estimate feeding value, 5 of the 10 hybrids (Brulouis, Dea, Mohican, LG 22, Lorena), one improved control LG 11 bm3 (brown mid-rib 3) and the normal hybrid LG 11 were used. The control LG 11 bm3 was obtained after at least 7 back-crosses of each of the constituent inbred lines of the normal three-way hybrid LG 11. Two plots of 250 m\(^2\) were sown for each hybrid at the density of 85 000 plans ha\(^{-1}\). They were ensiled according to their earliness and micro-silos were made (1 silo per replicate). Voluntary intake, digestibility in vivo of organic matter and digestibility of cellulose were evaluated with 8 sheep for each silo. Cellulose was estimated by the Weende method. Net energy was calculated by the INRA method (Jarrige, 1978) and was expressed in feed units for milk (UFL) and for meat (UFV), where 1 UF is equivalent in feeding value to 1 kg of barley.

Results and discussion

Patterns of dry-matter production Yield, dry-matter content and leaf area index are summarized in Table 1. The course of whole-plant yield through the season for each maturity group (average of early, medium-early... genotypes) is plotted in Figure 1. The course of leaf area index for the same groups is shown in Figure 2.

There were no differences in mass of whole plant between hybrids during the first 20 days.
Fig. 2. Course of leaf area index for each group of genotypes.

Table 1. Comparison of early and late genotypes for weight of whole plant and stover, and leaf area index at four dates.

<table>
<thead>
<tr>
<th>Sample no</th>
<th>Brulouis</th>
<th>Leader</th>
<th>Baron</th>
<th>Dea</th>
<th>Mohican</th>
<th>LG22</th>
<th>Roc</th>
<th>Tador</th>
<th>Lorena</th>
<th>SNH 931</th>
<th>F genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Av. wt of whole plant (g)</td>
<td>82.5</td>
<td>97.6</td>
<td>120.1</td>
<td>115.5</td>
<td>119.9</td>
<td>124.2</td>
<td>122.1</td>
<td>146.4</td>
<td>134.3</td>
<td>93.2</td>
<td>3.2* 1.8 NS</td>
</tr>
<tr>
<td>Content of dry-matter in whole plant (%)</td>
<td>18.0</td>
<td>17.3</td>
<td>16.9</td>
<td>17.4</td>
<td>14.2</td>
<td>14.1</td>
<td>13.1</td>
<td>12.5</td>
<td>11.5</td>
<td>11.8</td>
<td>4.4** 19.8** 16.3** 32.5**</td>
</tr>
<tr>
<td>Av. wt of stover per plant (g)</td>
<td>61.9</td>
<td>59.5</td>
<td>111.5</td>
<td>79.7</td>
<td>153.7</td>
<td>114.8</td>
<td>146.2</td>
<td>158.9</td>
<td>162.1</td>
<td>191.3</td>
<td>48.6** 58.5**</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample no</th>
<th>Content of dry matter in stover</th>
<th>Leaf area index</th>
<th>Number of leaves at mid-silking</th>
<th>Date of mid-silking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brulouis</td>
<td>18.4</td>
<td>3.3</td>
<td>3.3</td>
<td>9.8</td>
</tr>
<tr>
<td>Leader</td>
<td>18.7</td>
<td>3.9</td>
<td>3.1</td>
<td>3.1</td>
</tr>
<tr>
<td>Baron</td>
<td>19.8</td>
<td>4.7</td>
<td>4.3</td>
<td>4.5</td>
</tr>
<tr>
<td>Dea</td>
<td>20.0</td>
<td>3.9</td>
<td>3.2</td>
<td>3.4</td>
</tr>
<tr>
<td>Mohican</td>
<td>18.8</td>
<td>6.0</td>
<td>6.1</td>
<td>6.3</td>
</tr>
<tr>
<td>LG22</td>
<td>21.8</td>
<td>5.2</td>
<td>6.1</td>
<td>5.5</td>
</tr>
<tr>
<td>Roc</td>
<td>22.0</td>
<td>5.8</td>
<td>6.1</td>
<td>6.0</td>
</tr>
<tr>
<td>Tador</td>
<td>21.2</td>
<td>6.2</td>
<td>6.6</td>
<td>6.1</td>
</tr>
<tr>
<td>Lorena</td>
<td>21.5</td>
<td>6.4</td>
<td>7.2</td>
<td>7.1</td>
</tr>
<tr>
<td>SNH 931</td>
<td>19.9</td>
<td>6.8</td>
<td>7.7</td>
<td>7.7</td>
</tr>
<tr>
<td>F genotypes</td>
<td>6.5**</td>
<td>21.2**</td>
<td>35.2**</td>
<td>42.7**</td>
</tr>
</tbody>
</table>
after mid-silking of the early ones (Table 1; Figure 1). Differences first appeared at the time of silking of very late hybrids and then increased, the yield of the 4 earliest hybrids remaining constant. Late hybrids had a higher plant mass not only in a medium-south area, but also in northern areas (Ashoowalia, 1973; Derieux, 1983). One medium-late hybrid (Mohican) behaved like the latest ones for plant mass and number of leaves, probably partly because of ear prolificacy and physiology (Philipppe, 1980; Boyat et al., 1983; Barrière et al., 1984).

Changes in leaf area index were as previously described for maize (Eik & Hanway, 1965; Dale et al., 1980; Blanchet et al., 1982). At the time of silking of early hybrids, the index of later hybrids was about twice as high, even if mass of dry matter was similar. The interception of incident radiation by maize with an index of 3.5 is nearly 92%, according to the equation of Bonhomme et al. (1982) \( I = 1 - e^{-0.7 \text{ LAI}} \), and about 98% with an index of 6.0. This quite small difference, compared to all possible limiting environmental factors, perhaps partly explains the lack of yield differences between hybrids. This may also be related to Sanderson's observations (Daynard & Tollenaar, 1983) that there are no differences in growth rate in August between hybrids ranging in index from 2.1 to 5.7. In our trial, lack of differences for an index of 2.1 would, however, be difficult to explain in the same way. The better yields of later hybrids are not explained here by higher index. During August and September, the higher photosynthetic efficiency of physiologically younger leaves may be the main explanation (Ruget, 1981; Tollenaar & Migus, 1984). At the end of the season, the forging ahead in yield of later hybrids could be explained by their better leaf area duration, even though photosynthetic active radiation would then be decreasing (Varlet-Grancher, 1982). Comparison of the index of early and late genotypes at the end of the season clearly shows that the decrease of the index is genetically controlled, since this rapid decrease occurs only on physiologically old hybrids, and not exactly in the same way for every hybrid.

**Evaluation of feeding value with sheep**  There was no difference between hybrids for voluntary intake by sheep, despite large differences in proportion of grain in silage. This may be related to the observations of Gallais et al. (1981) that intake is not correlated with proportion of grain but with stover quality. Cellulose content was higher in later hybrids, and digestibility of cellulose was the same for all hybrids except LG 11 bm3 with a higher value. Digestibility of organic matter increases with earliness and may be related at least as much to a higher degree of lignification of later hybrids as to a higher grain content of earlier hybrids. Mohican and LG 22 have the same digestibility, despite 8% more grain in LG 22, indicating the role of stover in forage maize quality. Net energy content was high in earlier hybrids (Table 2) measured with UFL fodder units, ranging from 0.84 to 0.87 UFL/kg for early hybrids and 0.89 UFL/kg for LG 11 bm3, 0.05 UFL/kg better than normal LG 11; the value was about 0.10 UFL/kg less in medium-late hybrids than in earlier, and about 0.15 UFL/kg less for a very late hybrid such as Lorena. Nevertheless, according to the better yield of later genotypes, 22 t/ha against 12 t/ha, the yield of net energy was about 50% higher for Lorena than for Brulouis or Dea, and so for LG 11 bm3. This last observation does not undermine the interest of bm3 material, that must be compared with normal hybrids of the same genetic background. Trials with dairy cows and steers show that the farmer could reduce the amount of energy-rich concentrates per animal by 1 to 1.3 kg/d when they supply bm3 hybrids rather than quasi-isogenic normal types, with a voluntary intake of bm3 silage 1-1.5 kg/d higher (Gallais et al., 1981; Barrière et al., 1985; Malterre et al., 1985; Hoden et al., 1985).
Table 2. Agronomic data and feeding value with sheep of early and late genotypes.

<table>
<thead>
<tr>
<th>Harvest date</th>
<th>Yield (t/ha)</th>
<th>DM content (%)</th>
<th>Grain content (%)</th>
<th>Intake per metabolic weight of sheep (g/kg%</th>
<th>Content of cellulose (%)</th>
<th>Digestibility of cellulose (%)</th>
<th>Digestibility of organic matter (%)</th>
<th>Net energy content (UFL/ha)</th>
<th>Net energy content (UFV/ha)</th>
<th>10^3 Fodder Yield/ha</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brulouis</td>
<td>09-28</td>
<td>11.8</td>
<td>36.3</td>
<td>52.5</td>
<td>47.7</td>
<td>17.5</td>
<td>47.1</td>
<td>71.7</td>
<td>0.87</td>
<td>0.81</td>
</tr>
<tr>
<td>Dea</td>
<td>10-04</td>
<td>12.2</td>
<td>31.8</td>
<td>55.1</td>
<td>45.5</td>
<td>17.4</td>
<td>47.6</td>
<td>71.5</td>
<td>0.86</td>
<td>0.80</td>
</tr>
<tr>
<td>Mohican</td>
<td>10-12</td>
<td>17.6</td>
<td>27.9</td>
<td>38.7</td>
<td>46.1</td>
<td>21.6</td>
<td>46.1</td>
<td>65.8</td>
<td>0.76</td>
<td>0.69</td>
</tr>
<tr>
<td>LG22</td>
<td>10-12</td>
<td>14.7</td>
<td>34.2</td>
<td>46.2</td>
<td>48.3</td>
<td>20.8</td>
<td>43.1</td>
<td>65.9</td>
<td>0.77</td>
<td>0.69</td>
</tr>
<tr>
<td>Lorena</td>
<td>10-24</td>
<td>22.3</td>
<td>27.4</td>
<td>33.5</td>
<td>46.1</td>
<td>25.5</td>
<td>48.2</td>
<td>62.9</td>
<td>0.71</td>
<td>0.64</td>
</tr>
<tr>
<td>LG11</td>
<td>09-28</td>
<td>13.4</td>
<td>34.6</td>
<td>50.5</td>
<td>52.6</td>
<td>17.7</td>
<td>44.5</td>
<td>69.9</td>
<td>0.84</td>
<td>0.78</td>
</tr>
<tr>
<td>LG11 bm3</td>
<td>09-28</td>
<td>11.3</td>
<td>34.0</td>
<td>48.7</td>
<td>47.5</td>
<td>18.3</td>
<td>57.2</td>
<td>73.4</td>
<td>0.89</td>
<td>0.84</td>
</tr>
<tr>
<td>F genotypes</td>
<td>–</td>
<td>28.3**</td>
<td>6.0</td>
<td>11.8**</td>
<td>0.6 NS</td>
<td>4.7*</td>
<td>0.4 NS</td>
<td>27.0*</td>
<td>30.5**</td>
<td>40.2**</td>
</tr>
</tbody>
</table>

NS: not significant. **: highly significant. *: significant.
Conclusions

The best yields of silage are not obtained from hybrids with a high proportion of grain. The physiology of a silage maize hybrid is not the same as that of a grain maize. So silage varieties must be specialized. Specific criteria must be applied early in a breeding programme for silage maize, because yield and maturity are largely determined by stover. Breeding efforts should be devoted to use of the yield potential of late material, the variability of stover for natural drying, the different ways to enhance feeding value in normal as in bm3 material. They must also be devoted to breeding varieties with a good lodging resistance, and, if possible, an improved protein content. If farmers are to optimize utilization of silage maize in relation to the performance of their herds and to the intake of silage desired, they need to base their choices on the net energy content and earliness of the maize hybrids.

References


Dry-matter content of stover as a selection criterion for silage maize

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Summary

We propose to use dry-matter content of stover, that is all the harvested aerial parts apart from the ear, as a selection criterion for silage maize. A high dry-matter content in stover helps the plant to reach harvest maturity for silage at an earlier stage of development, thereby creating room to employ breeding material of relative late maturity, this to maximize yield potential. Selection for dry-matter content in stover can be started in the breeding nursery on an individual-plant basis. A good indication of dry-matter content can be obtained by sampling the stalk section just above the ear.

Descriptors: silage maize, Zea mays L., breeding, dry-matter content, carbohydrate content, growth period, heritability

An early maize variety is characterized by early flowering, short plants and early maturity, in combination with a moderate yield. In general, this is true for an early silage variety too. However the criterion for harvest maturity of silage maize is its dry-matter content, which is not wholly determined by flowering date or by the grain maturity. In that dry-matter content manifests itself as an independent character, we find some scope to select for earliness without sacrificing yield. This paper will enlarge on our findings.

Figure 1 illustrates the increase in dry-matter content towards the end of the season. While there is a rapid increase of dry-matter content in the ear, that in stover lags behind. Stover includes the harvested aerial parts that remain after removal of the ear. It consists of the stalk, leaves, tassel, the ear shank and the ear husks. The dry-matter content of stover remains almost constant until the ear has reached dough ripeness, and then starts to increase. The dry-matter content of stover is the result of two counteracting forces. Moisture loss towards maturity increases it; translocation of assimilates from the stover to the ear decreases it (Struik, 1983). As these two processes, moisture loss and translocation, depend also on environmental conditions, the resulting dry-matter content of stover relative to ear varies with growing conditions.

The crop is ready for harvest when the dry-matter content of the whole crop, the combination of stover and ear, has reached a certain minimum. In the Netherlands 280 g/kg is generally considered acceptable. Depending on farming conditions, this figure may be somewhat different for other areas (Hunter, 1978).

Of interest here are the genetic differences in dry-matter content of the stover. Ebskamp (1980), working at the Dutch Variety Office, noted that the correlation between dry-matter content of the ear and that of stover is about zero. This observation was made within the
limited range of varieties offered for testing in the Netherlands. A correlation of zero implies that one can alter the dry-matter content of the stover without changing the ear maturity.

We studied the relationship between silking date, content of dry-matter in ear and in stover, using S1 families from different genetic backgrounds. All families were within the maturity range adapted to the Netherlands. The families were grown in 1984 at two sites, Rilland and Wouw, both in the southwest of the Netherlands. Dry-matter content of ear and stover were determined when the crop had reached silage maturity.

The negative correlation found between silking date and dry-matter content of ear \( r = -0.74 \) indicated, as would be expected, that early flowering was associated with low moisture in the ear and vice versa. The other two values, however, were somewhat surprising. A high dry-matter content of stover correlated with late flowering, \( r = 0.48 \), and in line with this there was a negative correlation between dry-matter content of ear and stover, \( r = -0.43 \). These results may have arisen because we used materials from very different backgrounds in our study. Even so, our data indicate that we may alter the dry-matter content of stover without affecting flowering date or grain maturity.

An increase in dry-matter content of stover gives a corresponding increase in that of the total crop (Figure 1). This means we may shorten the time needed to reach harvest maturity, the date at which the crop has reached the critical dry-matter content needed for ensiling. Harvest maturity may decrease without influencing grain maturity, or even without affecting yield potential.
In a selection programme for silage maize, emphasis is normally placed on the combination of high yield of total dry-matter and high dry-matter content. This emphasis leads to indirect selection for high dry-matter content of stover.

More efficient would be that direct selection for a high content of stover dry-matter should start in the breeding nursery. In developing inbred lines, the flowering date, often used as a measure for maturity, works unsatisfactorily, because emphasis on early flowering severely restricts yield potential. A better indication for maturity is given by dry-matter content of stover at a given date for silage maturity. This asks for a non-destructive procedure to determine dry-matter content, because we want to preserve the still immature ear. We adopted the following method. We cut off the stalk just above the main ear. The dry-matter content of the first node above the ear node, together with parts of the adjoining internodes gives a good measurement of dry-matter content of stover. We found a correlation of \( r = 0.869 \) or \( r^2 = 0.76 \) between dry-matter content of the stalk section and the dry-matter content of stover. So it is possible to select plants individually for dry-matter content of stover.

Often selection by individual plants is not very effective because of the low heritability achieved. In our study, however, we found that heritability of dry-matter content in stover estimated on the basis of individual plants is rather high, \( h^2 = 0.34 \). This indicates that individual selection is feasible. The high heritability resulting from our data also points to the high genetic variation within the material under study. If we select within a more elite, narrower genetic stock, the heritability should be lower.

One final point. A high content of stover dry matter could be the result of early senescence. When leaves and stalk die off prematurely, this should have a positive effect on dry-matter content. The use of dry-matter content as a criterion would then lead to the selection of a very poor-looking crop. This is not what happens. We have to measure the dry-matter content of stover at a stage early enough that all plants are still green and healthy, without any sign of stalk rot (\textit{Fusarium} sp.). Our observation is that plants with a high dry-matter content in stover at this stage have a better than average capacity to stay green and have an above-average resistance to stalk rot. A high dry-matter content in stover indicates a high content of carbohydrates. It is well known that early senescence and susceptibility to stalk rot is caused by lack of carbohydrates in the stalk.

To clarify the argument once more. In a mature crop, a high dry-matter content in stover indicates premature death. At an earlier stage of development with a dry-matter content in ear of between 300 and 500 g/kg, a high dry-matter content in stover could be a sign of health.

References


Selecting hybrids for silage maize production: a Canadian experience

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Summary

There have been several attempts to develop superior hybrids for silage production. Material radically different from the conventional grain hybrid has been disappointing. Examination of current information suggests that hybrids developed specifically for whole-plant silage production will not look materially different from a grain hybrid. Major emphasis in selection will be on yield of whole-plant dry-matter (DM) with less emphasis on grain yield, stalk lodging resistance, plant barrenness and maturity.

In Canada, hybrids currently on the market were developed for grain production. A study determined the relationship between grain yield after grain maturity and yield and quality of whole-plant DM at the silage-harvesting stage. There was a significant linear relationship between grain yield and silage yield. However the relationship was not complete enough to permit reliable selection of hybrids for silage production based on grain yield.

Preliminary results of a recurrent selection programme aimed at development of improved germplasm for silage production are reported. Selection of S1 lines based on whole-plant yield in silage trials was more effective in increasing yield of whole-plant DM than selection of S1 lines based on grain yield in grain-production trials.

Descriptors: silage maize, Zea mays L., silage production, silage hybrids, recurrent selection, digestibility

Background

Maize is a major forage species. As a forage, it is used primarily in the production of whole-plant maize silage. Canada currently has in the order of 400 000 ha of maize grown for silage. Throughout North America and Europe, the primary objectives in the production of maize for silage have been high yield of dry matter (DM), high quality for ruminants and a high enough DM content to ensure proper ensiling with minimum losses as well as high DM intake.

Until recently, maize breeding has concentrated on the development of germplasm and hybrids for grain production. To a large extent, production practices have also been those developed for grain production.

At Guelph, our initial silage research examined field production practices for silage maize relative to grain production. It is not my intention to spend much time reviewing the research on silage production practices, except to note that the Ontario Government recommends higher planting densities and longer-season hybrids if the maize is being grown for silage. Our present approach suggests that planting densities about 20% higher than recommended for grain production will result in increased yields of DM with little or no loss of quality. In terms of hybrid maturity, we suggest that farmers consider growing hybrids for silage that are about
200 Ontario Corn Heat Units (Brown, 1978) later than recommended for grain. This equates with a rating of FAO 300 rather than FAO 200 or about two weeks in the autumn. The other suggestion we make is that it is more important for a farmer to plant his grain maize early than his silage maize. However delayed planting of maize for silage production results in reduced yields of DM, increases risk of immature maize and delays harvest in the autumn. The reason for this recommendation is that delayed planting reduces grain yield relatively more than whole-plant yield. In addition, the problem of delayed maturity for grain is more critical since the grain component is the last to develop. Any reduction in grain yield, therefore, results in a loss of the only component to be harvested. This is not true for whole-plant yield where the effects of delayed maturity on grain yield are counterbalanced by the effect on stover yield (Daynard & Hunter, 1975).

Recent research has examined the effect of stress on the performance of maize when grown for silage compared to grain production (Mbewe & Hunter, 1985). Shading stress at each of three stages of development (vegetative, reproductive and grain filling) all resulted in a reduction in yield of whole-plant DM relative to an unshaded control. The reduction in yield was similar in magnitude for all three shade treatments. By contrast, grain yield was reduced far more when the stress was applied at the reproductive stage compared to stress at the vegetative or grain-filling stages. For whole-plant yield, there was no similar reduction from shading during reproduction because a higher stover yield compensated for the reduction in grain yield.

Having briefly discussed some of the production practices and the stress response of maize grown for silage production, the balance of this paper will examine the role of genotype in silage production. The brief discussion on production practices supplies some information on what cultural practices will be used, at least at present, for maize grown for silage.

**Silage ideotype**

In a search for better 'silage hybrids', considerable research effort has been devoted to examining 'different' maize types. In search of higher DM yields, researchers have examined tillering maize (Freyman et al., 1973), extremely late maturing maize that is very tall and leafy (Hunter & Kannenberg, 1970) and barren or male-sterile plants (Perry & Caldwell, 1969). None of these approaches has shown much promise of increasing DM yields. In addition, maize genotypes producing little or no grain (very late maturing or barren or male-sterile maize) have been found to have moisture contents higher than desirable for good silage. Attempts to provide silage genotypes with better quality have included the use of dwarfing genes (Byers et al., 1965) and tillering germplasm (Bowden et al., 1973). Once again, no clear advantages were demonstrated. An exception to this is the feeding value of brown-midrib material (i.e. maize containing the bm3 gene). Such material has been demonstrated to be lower in whole-plant lignin content (Muller et al., 1971). The result has been improved feeding performance as demonstrated by, for example, Colenbrander et al. (1975). Unfortunately, the field performance of brown-midrib germplasm has been found to be inferior to normal material.

In summary, at present there do not seem to be radically different maize genotypes that will make a major contribution to improving yield or quality of maize grown for whole-plant silage. If we are not looking for anything radically different, what then are we looking for?

There is not a great deal of information about what a silage hybrid should be. As stated earlier, we know the objectives, but how to meet these objectives in a breeding programme
has been less clear. In North America, most breeders do not select material for silage performance but rather rely on the view that the best grain-yielding hybrids are the most suitable for silage. This view is based primarily on work in the United States in the 1930s and 1940s (e.g. Nevens, 1933). These early comparisons concluded that in general the best grain genotype also produced high-yielding high-quality forage at a DM content suitable for ensiling. The view was supported by the fact that the grain was high in quality relative to the stover. Maize breeders could also have accepted this premise because it eliminated the time and expense of separate breeding and evaluation programmes for silage maize. This would be a major consideration in regions where silage maize constitutes a small proportion of the total maize area; resources are better spent on evaluation for grain yield.

In many of the shorter-season areas into which maize has recently expanded, silage maize plays a greater role. In some areas, almost all the maize is used for silage production. Maize researchers in such areas are questioning the view of selecting the best grain hybrids and using them for silage. For example, in France, Gallais et al. (1976) found no relationship between the proportion of grain in hybrids and total DM yield. They did find differences between hybrids for yield of forage dry-matter. Their conclusion was that all parts of the plant must develop to the full in order to obtain highest yields. Stalk height and diameter and leaf size have been reported to be related to yields of forage dry matter (Gallais et al., 1976; Craig, 1966). However it would not be a suitable strategy to select for these traits alone without careful monitoring of maturity or else the selections would undoubtedly reflect maturity shifts as well as gains in DM yield. In shorter-season areas, this would result in low content of dry matter in forage through reduced grain content.

Under Canadian climatic conditions, the most direct approach to obtaining a DM content acceptable for making good whole-plant silage is to grow genotypes that produce a fairly high proportion of grain. It is not necessary to wait for maximum grain production (black layer) in order to obtain a high enough DM content. By the time the plant has attained 85-90% of maximum grain yield, the content of dry matter in aerial parts will be close to 35% (Daynard & Hunter, 1975). At this stage, grain moisture will be about 44%. In other words, research supports the view that high grain content as such should not be the goal of silage production. However it is necessary to have a sufficient grain content to assure a DM content suitable for ensiling. In Canada, we normally think in terms of DM content of 35%, which would relate to 40%, plus grain content. In the short-season areas of Europe, DM contents in the range 28-30% might be considered a reasonable maturity target. This target can be achieved with a lower grain content (i.e. less mature material).

It is well documented that the longer maize remains in the field, the more subject it is to stalk lodging resulting from stalk-cell senescence and stalk rots (Twumasi & Hunter, 1982). In addition, maize grown under increasing stress has been demonstrated to be more susceptible to lodging. Higher planting densities result in increased stress and, therefore, increased susceptibility to lodging. Since maize for silage is harvested at an earlier date and at an earlier stage in development than maize for grain, there is less opportunity for serious lodging. Countering this tendency is increased stalk lodging resulting from higher planting densities often associated with silage production than with grain production. Taking these two factors into account, selection criteria in obtaining material specifically for silage production should not need the same selection pressure to obtain satisfactory stalk-lodging resistance as when maize is being selected for grain. However, I know of no research that clearly examines and supports this contention.

With the above background, it is useful to hypothesize what might constitute a good silage
hybrid. The picture being sketched is by no means complete. A silage hybrid, at first glance, will probably not look noticeably different from a grain hybrid. It will probably produce a single ear on a single stalk. The grain-to-stover ratio will be dictated more by maturity requirements than by yield or quality. The grain-to-stover ratio at harvest will be of the order of 0.65 or in areas of very short-season even less. Emphasis need not be placed on maximizing grain yield. There will be as much emphasis on stover yield as on grain yield, but more important the emphasis will be placed on high yield of whole-plant dry matter.

The emphasis on maturity of grain can be less rigid for silage since the grain need not reach physiological maturity before the end of the growing season. In addition, less emphasis is required on selecting against stalk lodging and plant barrenness. The reduced emphasis on stalk barrenness, lodging resistance, grain maturity and grain yield should improve progress in selecting for high whole plant yield. This will be especially true in short-season areas where selection for maturity and against stalk lodging are major factors for hybrids to be used for grain production.

Selection against root lodging and general plant health must be maintained or even increased when selecting maize for silage as compared to grain. The increased emphasis would be related to increasing stress resulting from the increased planting densities often used for silage maize.

To date, the portion of the picture dealing with quality is not well outlined. There is some evidence that variation exists for improved stover digestibility, but care must be taken not to simply confuse stalk digestibility differences with maturity differences. As with yield, it is whole-plant digestibility that is important. The potential for selecting for this trait is not clear. There is some indication that selection for higher protein content would be possible. However in the North American context, considerable effort in selecting for higher protein content is not warranted.

Grain or silage maize breeding

In Canada, all maize hybrids currently on the market were selected for grain yield traits only. It would be of value to estimate how much variation there is for traits of silage performance within these grain hybrids and whether their grain yield predicts their merit for silage production. Trials (Vattikonda & Hunter, 1983) conducted at two sites over two years, examined all hybrids currently recommended for grain production in the two areas involved. A total of 81 hybrids were tested over the two years. The range was examined of variation that existed between hybrids for whole-plant yield, maturity and quality. In addition, the relationship between whole-plant yield and quality when harvested at the correct stage for silage production was compared with a hybrid's performance for grain production.

The major conclusions from this study are:

1. There was a significant linear relationship between the performance of hybrids for grain yield and their silage yield. However the coefficients of determination (0.23 and 0.25) for sites 1 and 2, respectively) were not large enough to permit reliable selection of hybrids for silage production based on grain yield performance.

2. Content of grain DM provided a reasonable estimate of plant maturity for silage production. Coefficients of determination between grain DM content and whole-plant DM content were 0.71 and 0.53 for sites 1 and 2, respectively.

3. For yield of forage, there was at least as much hybrid variation on a relative basis, and twice the hybrid variation on an absolute basis as there was for grain yield.
4. There was considerable variation between hybrids for stover digestibility and stover lignin content at time of harvest for silage production. Less variation existed for protein content.

gestibility of the stover component. In addition, there was no correlation between whole-plant DM content and whole-plant digestibility. This suggests that it may be possible, given the narrow range of maturities examined, to obtain material with high forage quality independent of hybrid maturity and grain content.

6. Whole-plant digestibility was related to both the content of grain in the silage and to the digestibility of the stover component.

7. Plant characteristics such as plant height, stalk diameter and resistance to lodging were not strongly correlated with parameters of silage performance such as whole-plant yield and quality.

In summary, the findings of this study support the need for separate evaluation trials for maize grown for silage production as opposed to grain production. Largely as a result of this research, the Province of Ontario initiated a silage testing programme for maize in 1984. Factors evaluated include whole-plant yield; whole-plant maturity and quality as determined by in vitro dry-matter digestibility and protein content.

Breeding for silage hybrids

There is very little reported in the literature on the results of actual selection schemes aimed at selecting material for silage performance traits as opposed to grain traits. Research at Guelph is currently under way with the following objectives:

- to determine the progress that can be made when selection is based on silage performance parameters
- to determine whether the germplasm selected for improved whole-plant yield and/or quality is of greater utility for silage production than maize selected for grain yield
- to determine changes in plant characteristics associated with the selection of germplasm for silage production.

The selection scheme being employed involves SI per se recurrent selection utilizing two populations, CG Syn A and Wigor. CG Syn A is a Guelph population derived from early dent germplasm from North America and Wigor represents early flint germplasm from Europe. Selection in five directions has been imposed in the synthetic CG Syn A (Cycle 0). Selection was based on:

i) high whole-plant yield
ii) high whole-plant digestibility \textit{(in vitro)}
iii) low whole-plant digestibility \textit{(in vitro)}
iv) high yield of digestible dry matter
v) high grain yield.

For Wigor, selection is based on only two traits as follows:

i) high yield of digestible dry matter
ii) high grain yield.

The selections involving whole-plant yield and quality (CG Syn A Selections i, ii, iii and iv and Wigor, Selection i) were based on SI performance traits with a production scheme for silage production. The selections for grain yield were based on tests with cultural practices suitable for grain production.

To date, two cycles of recurrent selection have been completed. The cycle 0, I and II popula-
tions were compared for silage performance parameters in 1984 at two sites. In 1985, this research has been expanded considerably and the populations will be examined in detail for both grain yield under a grain-production scheme and silage yield under a silage-production scheme. Mean data from the two sites from 1984 for yield of whole-plant DM and for digestibility are presented in Tables 1 and 2, respectively. The information must be considered preliminary but it allows a general evaluation of the performance per se of the populations.

For the population CG Syn A, a higher-yielding population, there was little improvement in whole-plant yield at the silage harvest stage when the S1 lines were selected on the basis of grain yield per se in grain yield production trials. In contrast, selection of S1 lines based on yield of whole-plant DM or yield of digestible DM resulted in increased yield of whole-plant DM. For example, the CII material produced from selection of S1 lines for yield of digestible DM yielded 16% more whole-plant DM than when selection was based on grain yield. Selection for either high or low digestibility had little effect on whole-plant yield.

In the low-yielding Wigor population, selection of S1 lines on the basis of either increased grain yield in grain-production plots or increased yield of whole-plant digestible DM resulted in a marked increase in yield of whole-plant DM. However the increase was much more dramatic when selection was based on yield of whole-plant digestible DM. There was no meaningful change in digestible dry-matter content (Table 2). This was true even when selection was based strictly on content of digestible dry-matter.

Table 1. Yield of whole-plant dry matter (t/ha) of the C0, CI and CII population of material involved in an SI recurrent selection programme per se. Means of trials at two sites.

<table>
<thead>
<tr>
<th>Population</th>
<th>Selection criteria</th>
<th>Cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C0</td>
</tr>
<tr>
<td>CG Syn A</td>
<td>grain yield</td>
<td>10.7</td>
</tr>
<tr>
<td></td>
<td>yield of whole-plant DM</td>
<td>10.7</td>
</tr>
<tr>
<td></td>
<td>yield of whole-plant digestible DM</td>
<td>10.7</td>
</tr>
<tr>
<td></td>
<td>high digestibility</td>
<td>10.7</td>
</tr>
<tr>
<td></td>
<td>low digestibility</td>
<td>10.7</td>
</tr>
<tr>
<td>Wigor</td>
<td>grain yield</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>yield of whole-plant digestible DM</td>
<td>6.0</td>
</tr>
</tbody>
</table>

Table 2. Content (%) of whole-plant in vitro digestible dry matter of the C0, CI and CII populations of material involved in an SI per se recurrent selection programme. Means of trials at two sites.

<table>
<thead>
<tr>
<th>Population</th>
<th>Selection criteria</th>
<th>Cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C0</td>
</tr>
<tr>
<td>CG Syn A</td>
<td>grain yield</td>
<td>71.3</td>
</tr>
<tr>
<td></td>
<td>yield of whole-plant DM</td>
<td>71.3</td>
</tr>
<tr>
<td></td>
<td>yield of whole-plant digestible DM</td>
<td>71.3</td>
</tr>
<tr>
<td></td>
<td>high digestibility</td>
<td>71.3</td>
</tr>
<tr>
<td></td>
<td>low digestibility</td>
<td>71.3</td>
</tr>
<tr>
<td>Wigor</td>
<td>grain yield</td>
<td>72.0</td>
</tr>
<tr>
<td></td>
<td>yield of whole-plant digestible DM</td>
<td>72.0</td>
</tr>
</tbody>
</table>
In summary this information leads to the following tentative conclusions:
- Selection of SI lines based on whole-plant yield in silage trials is more effective in increasing whole-plant DM yield than selection of SI lines based on grain production.
- There appears to be a limited prospect of selecting for differences in whole-plant digestibility.
- Selection based on either yield of digestible DM or on whole-plant DM yield leads to similar gains in whole-plant DM yield. This probably reflects the lack of differences in content of digestible dry-matter in the material tested.

References

Nevens, W.B., 1933. Types and varieties of corn for silage. University of Illinois Agricultural Experiment Station Bulletin 391.
Analysis of factorial crosses between flint and dent maize inbred lines for forage performance and quality traits

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Summary

Sixty-six F1 forage maize (*Zea mays* L.) hybrids from an incomplete factorial mating design among 11 flint and 11 dent inbred lines of current European breeding materials were evaluated in 1983 and 1984 at three sites in West Germany for the following silage traits: plant height; dry matter content (DMC) of ear, stover and forage; dry matter yield (DMY) of grain, stover and forage; crude protein content (CPC) of grain and stover; metabolizable energy content (MEC) and yield (MEY) of stover. Highly significant differences among hybrid means and interactions with environment were found for all traits. Variation among hybrids was generally smaller for the quality traits (CPC, MEC) than for the agronomic traits. Variances of general combining ability were significant and greater than of specific combining ability for most traits. Heritability coefficients on an entry mean basis ranged from 0.61 to 0.96, being lowest for DMY of grain, medium for CPC and MEC and highest for the remaining traits. DMY and DMC of forage had a strong negative relationship, whereas correlations among quality traits and between quality and agronomic traits were generally moderate to low. Our results suggest that variation in DMY of stover offers great potential for improving total DMY. MEY of stover was mainly determined by DMY and less by MEC.

Descriptors: maize, *Zea mays* L., factorial crosses, combining ability, heritability, genetic correlations, silage production, silage quality

Introduction

High dry matter yield (DMY), high feeding quality for ruminants and sufficiently high dry matter content (DMC) to ensure proper fermentation and high intake by livestock are regarded as the primary objectives in silage maize production (Gallais et al., 1976; Hunter, 1978). Most breeders have concentrated on developing hybrids for grain production, assuming that high-grain-producing genotypes are also high producers for forage. Recent research, however, suggests that the non-grain portion of the maize plant offers substantial opportunity for improvement in yield and quality (Gallais et al., 1981).

Use of the brown midrib mutant (*bm3*) was proposed as one way to increase the digestibility of maize stover. But since it also considerably reduces DMY, Miller et al. (1983) and Lee & Brewbaker (1984) concluded that ordinary maize populations should offer more potential for silage breeding programmes. Highly significant genetic differences in the stover quality of ordinary hybrids were reported by Gallais et al. (1976) and Deinum & Bakker (1981).

The present study was conducted (i) to analyse the genetic variability in DMY and several quality traits of grain and stover in current breeding materials adapted to north-west Europe.
and (ii) to estimate genetic parameters in this material relevant to the development of optimal breeding strategies to improve those traits.

Materials and methods

The experimental materials consisted of 66 F1 maize hybrids established by crossing 11 flint with 11 dent inbred lines according to an incomplete factorial mating design (Melchinger, 1984), each line being involved in 6 crosses. Both parental groups represent samples of modern European breeding materials (no brown midrib nor IHP) of early maturity (FAO 200-300). The hybrids were grown in a simple lattice design in 1983 and 1984 at three sites (Stuttgart-Hohenheim, Soellingen, Giessen), representing a broad range of agro-ecological conditions within West Germany. Density ranged between sites from 8 to 10 plants per square metre.

Data was recorded for plant height, DMC of ear and stover, and DMY of grain, stover and forage. Crude protein content (CPC) of grain and stover was estimated for ground samples by near-infrared reflectance (Hymowitz et al., 1974; Marten et al., 1983). Metabolizable energy content (MEC) of stover was measured in vitro by the recently developed gas-production method of Menke et al. (1979) and used to calculate the metabolizable energy yield (MEY) of stover. These measurements were done in the laboratory of Professor Dr. K.H. Menke (University of Hohenheim).

Combined analyses of variance and estimation of phenotypic and genotypic correlations were performed by established procedures (Cochran & Cox, 1957; Mode & Robinson, 1959). Heritability ($h^2$) on an entry mean basis was calculated as the ratio of genotypic to phenotypic variance of hybrids. Variances of general combining ability (g.c.a.) and specific combining ability (s.c.a.) were estimated by Method 3 of Henderson (1953) as outlined by Melchinger (1984).

Results

DMC of ear and stover as well as DMY of grain were consistently higher in 1983 than in 1984 when temperatures were below average (Table 1). At Soellingen, killing frost occurred before the stover harvest in 1983, explaining the high DMC of stover and forage. No satisfactory maturity was reached at Giessen in 1984. Otherwise, DMC of forage was within the range recommended by animal nutritionists. DMY of stover was stabler between years and sites than DMY of grain. MEC of stover hardly varied between sites, despite different maturity.

The combined analyses of variance (not given) yielded highly significant ($P < 0.01$) mean squares for hybrids and interactions of hybrids with sites for all traits. The range and coefficient of variation (c.v.) for DMC of ear and stover revealed considerable differences in maturity among hybrids (Table 1). DMY of stover varied widely between hybrids, about twice as much as DMY of grain. Variation in CPC was small for both grain and stover. In contrast to MEC which showed the lowest c.v., MEY of stover had the highest c.v. and the widest range of all traits.

Variances of g.c.a. were highly significant for most traits, exceeding twice their respective standard errors apart from few exceptions (Table 2). The variances of s.c.a. were generally less than half of those of g.c.a., yet with one exception highly significant. Variances of interactions of g.c.a. effects with site were highly significant for all traits but small relative to variances of g.c.a. Only for DMY and CPC of grain were variances of interactions considerably

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Table 1. Means of 66 forage maize hybrids for eleven agronomic and silage quality traits at six sites. Hoh, Hohenheim (Baden-Württemberg); Soe, Soellingen (Lower Saxony); Gie, Giessen (Hessen): DMC in fresh matter; CPC and MEC in dry matter.

<table>
<thead>
<tr>
<th>Year</th>
<th>Site</th>
<th>Plant height (m)</th>
<th>DMC (g/kg)</th>
<th>DMY (kg m⁻²)</th>
<th>CPC (g/kg)</th>
<th>MEC (MJ/kg)</th>
<th>MEY (MJ m⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>ear</td>
<td>stover</td>
<td>forage</td>
<td>grain</td>
<td>stover</td>
</tr>
<tr>
<td>1983</td>
<td>Hoh</td>
<td>1.66</td>
<td>454</td>
<td>222</td>
<td>305</td>
<td>0.551</td>
<td>0.657</td>
</tr>
<tr>
<td></td>
<td>Soe</td>
<td>1.83</td>
<td>567</td>
<td>332</td>
<td>437</td>
<td>0.817</td>
<td>0.692</td>
</tr>
<tr>
<td></td>
<td>Gie</td>
<td>2.26</td>
<td>532</td>
<td>239</td>
<td>360</td>
<td>0.807</td>
<td>0.614</td>
</tr>
<tr>
<td>1984</td>
<td>Hoh</td>
<td>2.06</td>
<td>362</td>
<td>205</td>
<td>251</td>
<td>0.435</td>
<td>0.834</td>
</tr>
<tr>
<td></td>
<td>Soe</td>
<td>1.90</td>
<td>476</td>
<td>253</td>
<td>335</td>
<td>0.513</td>
<td>0.556</td>
</tr>
<tr>
<td></td>
<td>Gie</td>
<td>2.17</td>
<td>311</td>
<td>174</td>
<td>210</td>
<td>0.284</td>
<td>0.662</td>
</tr>
<tr>
<td>Overall mean</td>
<td>1.98</td>
<td>450</td>
<td>237</td>
<td>316</td>
<td>0.568</td>
<td>0.669</td>
<td>1.380</td>
</tr>
</tbody>
</table>

Range among hybrids

maximum: 2.19 | 528 | 314 | 387 | 0.643 | 0.852 | 1.616 | 113.1 | 60.9 | 8.89 | 7.72 |
minimum: 1.74 | 354 | 205 | 266 | 0.466 | 0.444 | 1.127 | 92.5  | 45.9 | 7.87 | 3.63 |
I.s.d. 0.05: 0.08 | 24 | 27 | 24 | 0.088 | 0.076 | 0.106 | 6.2   | 5.2  | 0.32 | 0.73 |
c.v. (%): 4.8 | 4.1 | 10.2 | 9.2 | 7.8 | 14.1 | 7.2 | 4.1 | 6.9 | 3.2 | 15.7 |
Table 2. Components of variance ($\sigma^2$) and heritability ($h^2$) for eleven agronomic and silage quality traits determined from the combined analyses of variance of 66 forage maize hybrids grown at six sites. Standard error in parenthesis. *, $P \leq 0.05$; **, $P \leq 0.01$.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Plant height</th>
<th>DMC $\times 10^2$</th>
<th>DMY $\times 10^4$</th>
<th>CPC $\times 10^2$</th>
<th>MEC in stover</th>
<th>MEY of stover</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ear</td>
<td>stover</td>
<td>forage</td>
<td>grain</td>
<td>stover</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(20.5)</td>
<td>(3.9)</td>
<td>(1.0)</td>
<td>(1.6)</td>
<td></td>
</tr>
<tr>
<td>$\sigma^2_{S_{e.g.a.}}$ (flint)</td>
<td>46.6**</td>
<td>9.0**</td>
<td>2.2**</td>
<td>3.6**</td>
<td>6.2**</td>
<td>35.9**</td>
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<tr>
<td></td>
<td>(20.5)</td>
<td>(3.9)</td>
<td>(1.0)</td>
<td>(1.6)</td>
<td>(4.5)</td>
<td>(16.6)</td>
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<td></td>
<td></td>
<td></td>
<td>(16.0)</td>
<td></td>
</tr>
<tr>
<td>$\sigma^2_{S_{e.g.a.}}$ (dent)</td>
<td>45.3**</td>
<td>9.0**</td>
<td>2.6**</td>
<td>4.5**</td>
<td>-0.4</td>
<td>42.8**</td>
</tr>
<tr>
<td></td>
<td>(20.3)</td>
<td>(3.9)</td>
<td>(1.2)</td>
<td>(2.0)</td>
<td>(1.3)</td>
<td>(19.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(23.2)</td>
<td></td>
</tr>
<tr>
<td>$\sigma^2_{S_{g.e.c.a.}}$</td>
<td>8.1**</td>
<td>1.2**</td>
<td>0.4**</td>
<td>0.7**</td>
<td>4.1**</td>
<td>13.6**</td>
</tr>
<tr>
<td></td>
<td>(2.6)</td>
<td>(0.3)</td>
<td>(0.2)</td>
<td>(0.2)</td>
<td>(1.5)</td>
<td>(4.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(5.1)</td>
<td></td>
</tr>
<tr>
<td>$\sigma^2_{E \times S_{e.g.a.}}$ (flint)</td>
<td>8.0**</td>
<td>2.3**</td>
<td>0.7**</td>
<td>0.9**</td>
<td>20.0**</td>
<td>7.3**</td>
</tr>
<tr>
<td></td>
<td>(2.6)</td>
<td>(0.5)</td>
<td>(0.3)</td>
<td>(0.3)</td>
<td>(4.5)</td>
<td>(2.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(6.9)</td>
<td></td>
</tr>
<tr>
<td>$\sigma^2_{E \times S_{g.e.c.a.}}$ (dent)</td>
<td>12.5**</td>
<td>1.3**</td>
<td>1.4**</td>
<td>1.0**</td>
<td>12.0**</td>
<td>6.4**</td>
</tr>
<tr>
<td></td>
<td>(3.4)</td>
<td>(0.3)</td>
<td>(0.4)</td>
<td>(0.3)</td>
<td>(2.9)</td>
<td>(2.5)</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(3.6)</td>
<td></td>
</tr>
<tr>
<td>$\sigma^2_{E \times S_{g.e.c.a.}}$ (flint)</td>
<td>4.0</td>
<td>0.6**</td>
<td>1.1**</td>
<td>1.3**</td>
<td>3.6**</td>
<td>2.7**</td>
</tr>
<tr>
<td></td>
<td>(2.8)</td>
<td>(0.2)</td>
<td>(0.3)</td>
<td>(0.3)</td>
<td>(1.7)</td>
<td>(3.4)</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>(6.3)</td>
<td></td>
</tr>
<tr>
<td>$\sigma^2_{e}$</td>
<td>45.3</td>
<td>1.9</td>
<td>4.1</td>
<td>2.7</td>
<td>25.2</td>
<td>59.8</td>
</tr>
<tr>
<td></td>
<td>(2.4)</td>
<td>(0.1)</td>
<td>(0.2)</td>
<td>(0.1)</td>
<td>(1.3)</td>
<td>(3.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(6.4)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.8)</td>
<td>(1.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.0)</td>
<td>(2.6)</td>
</tr>
<tr>
<td>$h^2$ (%)</td>
<td>91.9</td>
<td>95.5</td>
<td>84.1</td>
<td>91.0</td>
<td>61.0</td>
<td>91.4</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>85.0</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>71.4</td>
<td>75.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>81.5</td>
<td>91.2</td>
</tr>
</tbody>
</table>
larger than those of g.c.a. and s.c.a..

Heritability ($h^2$) ranged from 0.61 to 0.96 (Table 2). DMY of grain was the least heritable trait; DMY and MEY of stover, and DMC of ear and stover showed high heritability. That of quality traits (CPC, MEC) was moderate.

Phenotypic and genotypic correlations were almost the same for most trait combinations (Table 3). Genotypic correlations were significant for DMC of ear with all other traits. Coefficients were highly negative with DMY and MEY of stover and moderately positive with DMY of grain. DMC of stover displayed moderate negative correlations with DMY and MEY of stover and CPC of grain. DMY of grain was negatively correlated with CPC of grain. DMY of stover showed high positive correlations with DMY of forage, MEY of stover, and plant height. Correlations of MEC of stover with other traits were moderate to low, apart from a higher value of MEY.

Discussion and conclusions

Our data reinforces the view that substantial genetic variation for stover yield and quality exists in ordinary breeding materials. The wide range in DMY of stover (Table 1) is consistent with results of a study by Mann et al. (1981). The relative range in MEC of stover (11%) was about twice as great as that in stover digestibility reported previously for hybrid varieties (Gallais et al., 1976; Wermke & Zimmer, personal communication, 1983) and experimental hybrids (Deinum & Bakker, 1981) of similar maturity. In comparison, experiments with the brown midrib mutant (bm3) yielded a relative increase in stover digestibility of 12% (Gallais et al., 1980).

The variance of g.c.a. considerably exceeded the variance of s.c.a. for all agronomic and quality traits, except DMY of grain (Table 2). Any recurrent selection procedure making use of the additive genetic variance should therefore be effective for improving stover yield and quality (MEC, CPC). Previous reports on grain maize confirm the predominance of additive variance for CPC of grain and stover (Pollmer et al., 1978). In contrast to our findings, Roth et al. (1970) established in a single-environment experiment that variance of g.c.a. and s.c.a. was similar for stover digestibility.

The low heritabilities for DMY and CPC of grain resulted from significant genotype-site interactions on account of extremely diverse weather in the two years of testing. All entries reached satisfactory maturity in 1983, whereas in 1984 with a shortened grain-filling period, later maturing hybrids could not reach their potential yield and had higher CPC than earlier maturing ones. Estimates of heritability for the other traits were exceptionally high. This suggests that only a few test environments are required to identify genotypes with improved stover yield and quality.

Sufficient DMC in forage represents another important criterion in selecting silage maize. The strong negative correlation between DMC and DMY of whole plant (Table 3) is of particular significance in that it impedes progress in selecting for high-yielding varieties.

In the literature (Hunter, 1978), DMC of ear and proportion of ear are generally considered as the main components determining DMC of whole plant. However in the present material, a closer relationship existed with DMC and DMY of stover. Interestingly enough, DMC of ear and stover were hardly correlated.

Genotypic correlations of DMY of forage were highly positive with DMY of stover and zero with DMY of grain. This indicates that differences in stover yield largely accounted for the observed variation in total DMY. For individual components, DMY of grain and stover dis-
Table 3. Phenotypic (above diagonal) and genotypic (below diagonal) correlations among eleven agronomic and silage quality traits determined from the combined analysis of 66 forage maize hybrids grown at six sites. *, ** Phenotypic correlation was significant at probability 0.05 and 0.01, respectively, and the genotypic correlation exceeded twice and three times its standard error, respectively.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Plant height</th>
<th>DMC</th>
<th>DMY</th>
<th>CPC</th>
<th>MEC in stover</th>
<th>MEY of stover</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ear</td>
<td>stover</td>
<td>forage</td>
<td>ear</td>
<td>stover</td>
</tr>
<tr>
<td>Plant height</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMC ear</td>
<td>-0.39**</td>
<td>-0.32*</td>
<td>-0.49**</td>
<td>-0.05</td>
<td>0.66**</td>
<td>0.67**</td>
</tr>
<tr>
<td>stover</td>
<td>-0.37**</td>
<td>0.28*</td>
<td>0.70**</td>
<td>0.48**</td>
<td>-0.65**</td>
<td>-0.46**</td>
</tr>
<tr>
<td>forage</td>
<td>-0.53**</td>
<td>0.72**</td>
<td>0.85**</td>
<td>0.46**</td>
<td>-0.76**</td>
<td>-0.54**</td>
</tr>
<tr>
<td>DMY grain</td>
<td>-0.01</td>
<td>0.51**</td>
<td>0.34**</td>
<td>0.58**</td>
<td>0.36*</td>
<td>0.16</td>
</tr>
<tr>
<td>stover</td>
<td>0.70**</td>
<td>-0.68*</td>
<td>-0.51**</td>
<td>-0.82**</td>
<td>-0.36*</td>
<td>0.89**</td>
</tr>
<tr>
<td>forage</td>
<td>0.72**</td>
<td>-0.54*</td>
<td>-0.37*</td>
<td>-0.62**</td>
<td>0.00</td>
<td>0.92**</td>
</tr>
<tr>
<td>CPC grain</td>
<td>0.41**</td>
<td>-0.35*</td>
<td>-0.59**</td>
<td>-0.66**</td>
<td>-0.53**</td>
<td>0.52**</td>
</tr>
<tr>
<td>stover</td>
<td>-0.16</td>
<td>-0.57*</td>
<td>0.12</td>
<td>-0.14</td>
<td>-0.26</td>
<td>0.10</td>
</tr>
<tr>
<td>MEC stover</td>
<td>0.01</td>
<td>-0.41**</td>
<td>-0.25</td>
<td>-0.42**</td>
<td>-0.35*</td>
<td>0.44**</td>
</tr>
<tr>
<td>MEY stover</td>
<td>0.61**</td>
<td>-0.70**</td>
<td>-0.51**</td>
<td>-0.82**</td>
<td>-0.40*</td>
<td>0.98**</td>
</tr>
</tbody>
</table>
played only a weak negative correlation. Similarly, Leask & Daynard (1973) failed to establish a significant correlation between grain and stover yield, whereas Gallais et al. (1981) reported positive correlations in some years.

The strong correlation between DMY of stover and plant height confirms observations of Gallais et al. (1976) that DMY of stover was closely correlated with plant height but less so with stalk diameter. Consequently, as taller genotypes tend to be more susceptible to root lodging, selection for increased stover yield needs to be accompanied by simultaneous selection for lodging resistance.

Although stover digestibility is known to decrease during maturation for a given genotype (Gallais et al., 1976), the estimated negative correlations between MEC of stover and DMC of ear and stover were rather low and thus should be of no concern to the plant breeder. The correlations between MEY of stover and its component traits reveal that DMY of stover accounted almost entirely for the variation in MEY, whereas differences in MEC only of secondary importance.

In conclusion, our study corroborates the importance of stover for both maximum DMY and sufficient DMC of forage. Since DMY of grain and stover were only weakly negatively correlated, it seems that improvement in stover yield could be made without unacceptable reduction in grain yield. The strong negative correlation between DMY and DMC of forage suggests the need to select only among genotypes of similar maturity (DMC of forage). DMC of ear turned out to be an inadequate criterion for maturity, rather DMC of stover has also be taken into account. Despite significant genotypic variation, the various quality traits (MEC of stover, CPC of grain and stover) seem to be of secondary importance compared to DMY and DMC of forage in developing improved hybrid varieties for silage use.

Acknowledgments

We are grateful to the firms KWS Kleinwanzlebener Saatzucht AG, Fr. Strube Saatzucht Soellingen and to Professor Schuster, University of Giessen, for providing facilities and assistance in establishing the crosses at Monsheim and the field trials at Soellingen and Giessen. This research was supported by Gemeinschaft zur Förderung der privaten deutschen landwirtschaftlichen Pflanzenzüchtung e.V. (GFP), Grant No M20/82.

References

Closing session
Report of the activities in the Maize Northern Committee

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Our main activity in the Maize Northern Committee is the Cooperative Eucarpia North Trial, which has a long-year tradition. After our discussions at the 1981 Congress in Montreux, Switzerland, we arranged our North Trial in 3 series:
- Series A: Silage maize trials
- Series B: Grain maize trials in less favourable climate
- Series C: Grain maize trials in more favourable climate.

In the 1983 trials there were 33 participating stations from 15 European countries, organized in the 3 series with 11 stations each. Each of the 33 participants could send 3 entries into the trials:
- 1 entry in Series A (to all 11 sites of Series A)
- 1 entry in Series B (to all 11 sites of Series B)
- 1 entry in Series C (to all 11 sites of Series C).

Together with the 3 control hybrids:
- Cargill Primeur 170
- Mutin KWS 181
- LG 11

there could be planted at each site a 6 × 6 simple lattice with 36 entries in 2 replicates (i.e. 72 plots per site). For different reasons, final evaluation considered only 20 locations:
- 6 sites in Series A
- 5 sites in Series B
- 9 sites in Series C

The results of 13 sites were not available or must be discarded.

This final evaluation was published for all participants in the 'Report on Cooperative Eucarpia North Trial 1983' in August 1984. Besides all available information on participants, entries and sites, this report includes the following results for all 3 series:
- extract of analysis of variance
- outlier test of Anscombe & Tukey
- results for each site
- means per site
- correlations and heritability
- LSD (5%) for each site
- mean values over sites
- phenotypic stability
- correlations between traits
The 1984 trials were organized on the same principle but with 36 participants from 15 European countries (in 3 series with 12 sites each). At each of the 36 sites, a randomized block design was planted with 40 entries in 2 replicates (i.e. 80 plots per site). The 40 entries per site consisted of 36 entries of the different participants (1 entry per participant) and the 4 control hybrids:
- Cargill Primeur
- Mutin KWS 181
- LG 11
- Dea P.3839.

Just as in the year before, only 23 sites could be included in the final evaluation:
- 8 sites in Series A
- 7 sites in Series B
- 8 sites in Series C.

The final evaluation of the 1984 trials was published for all participants in the 'Report on Cooperative Eucarpia North Trial 1984' in August 1985. Besides all available information on participants, entries and sites this report includes the following results for all 3 series:
- analysis of variance
- outlier test (Anscombe & Tukey)
- phenotypic stability
- correlation between traits
- results for each entry and site
- means for each site
- correlation, heritabilities and LSD for each site
- means over all sites
- scatter diagrams (for 2 traits each)
- scatter diagrams (for 3 traits each).

This year, 1985, the trials were organized as randomized block design with 40 entries in 2 replicates for each site (i.e. 80 plots per site). The number of participants was considerably less than in 1984, 18 stations in 11 European countries, so that the 3 series A, B and C include 6 sites each. But each participant could submit 6 entries to the trials:
- 2 hybrids into Series A (to all 6 sites of Series A)
- 2 hybrids into Series B (to all 6 sites of Series B)
- 2 hybrids into Series C (to all 6 sites of Series C).

As control hybrids, we used:
- Bema 210
- Mutin KWS 181
- Vispo KWS 233
- Dea P.3839.
During this growing season, all participating stations will observe and collect all data to allow final evaluation for the following major characteristics:

- **Yield**
  - of total dry matter
  - of kernels (corrected to 14% H₂O)
  - in Series A
  - in Series B and C

- **Maturity**
  - as content of total dry matter
  - as content of kernel dry matter
  - in Series A
  - in Series B and C

- **Standability**
  - as proportion of erect plants at harvest
  - in all series

- **Root lodging**
  - as noted during growth
  - in all series

- **Early vigour**
  - as noted in spring
  - in all series

- **Diseases**
  - as number of plants with smut
  - as number of plants with...
  - in all series
  - in all series

The final evaluation will be published for all participants in the 'Report on Cooperative Eucarpia North Trial 1985' in spring 1986.

**Acknowledgments**  All evaluations were calculated by Dr H.F. Utz, Institute of Plant Breeding and Population Genetics of the University of Stuttgart-Hohenheim with his computer programmes; we owe Dr Utz a debt of gratitude.
As it was already stated at the Warsaw congress, there had been some advance in several respects of this cooperation:
- the use of the same control varieties in all trials
- FAO grouping became more professional
- seed arrived more punctually.

In the last two years, comparative trials have been carried out in the ‘A’ and ‘B’ experimental groups in the FAO maturity groups 300 to 700.

In 1984, comparative trials were carried out at 19 sites. ‘A’ 14 sites; ‘B’ 5 sites. The lower number of ‘B’ indicates that fewer stations are interested in the 600-700 maturity group. However about half the maize-producing area in Southern Europe is of the 600 maturity group. The growing season of the hybrids is becoming shorter and shorter, though this tendency slowed down in the area of the Southern Committee in the last years.

In 1985, 19 members participated in the work of the comparative trials:

<table>
<thead>
<tr>
<th>Country</th>
<th>Sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulgaria</td>
<td>1</td>
</tr>
<tr>
<td>France</td>
<td>4</td>
</tr>
<tr>
<td>Italy</td>
<td>2</td>
</tr>
<tr>
<td>Hungary</td>
<td>2</td>
</tr>
<tr>
<td>Rumania</td>
<td>1</td>
</tr>
<tr>
<td>Soviet Union</td>
<td>5</td>
</tr>
<tr>
<td>Yugoslavia</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
</tr>
</tbody>
</table>

‘A’ trials were set out at 15 sites and ‘B’ trials at 4 sites.

Although the participants send the results to each other regularly, we have to confess that punctuality left much to be desired. In spite of this, the results of 1983 were summarized and sent to the participants at the end of 1984. Unfortunately, we received data only from 7 sites. In my opinion, nevertheless, supply of local data to all participants quickened the utilization of the trial results.

Also the data of the individual participants in 1984 trials were mostly reported directly to the other participants. Hopefully it contributed considerably to planning the trials both for 1985 and 1986. The participants are kindly requested to send the results of 1985 on time to the new president. With respect to the 1986 trials, it is advised to check the standards and to change them if necessary. Participants should not hesitate to change the present experimental design.
if it is not adequate any more. Further, it is stressed to send seeds on time. Some missing entries make comparison difficult and lead to unwanted delay in planting. These facts may result in the unreliability of the final data.
The last two years have been characterised by further increase in the importance of maize in Europe, especially for silage. Corn-cob mix is becoming major fodder in some countries. These facts are reflected in the choice of varieties for different purposes.

There is a tendency towards dominance of a few superior hybrids over large areas. Considerable progress is evident in the performance of hybrids. The movement of maize growing towards higher latitudes continues in spite of some unfavourable seasons, which seems to indicate an improvement in yield stability of early hybrids.

There is a constant increase in activity of our Section. The number of papers and communications is increasing with each meeting. This called for improving the efficiency of our meetings. The first step was made at the 12th meeting in 1983 in Warsaw: the monolingual system was adapted to reduce the cost and improve the efficiency of discussion. The next improvement was made at this meeting. Limitation of the number of papers presented at the plenary session enabled us to broaden discussions.

Cooperative trials constitute the second part of our activities. They were performed in the frames of the two maize Committees. Details have just been presented by their Chairman. The trials of the Northern Committee were better coordinated. In spite of the necessity of discarding some of the trials, the majority of them were included into the reports. The Southern committee based its work more on station-to-station exchange of data.

There is an evident decline in the activity of Sorghum group. There were very few papers on sorghum breeding at the 9th, 10th and 11th meetings and none at the 12th and 13th. It is a pity, because sorghum continues to be an important crop in the southern zone of Europe. We need to seek ways of reviving the Sorghum group.

Acknowledgment The chairmen of both Maize Committees, Dr. D. Alber and Dr. J. Németh, are sincerely thanked for their devoted work through several years for the Northern and Southern Committee, respectively.
Abstracts of posters
A maize variety with a large number of tillers and ears, and its potential for silage production

Bong-ho Choe and Hee-bong Lee

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A maize variety, MET, with a large number of tillers and ears was found in Korea as part of the result of a nationwide maize collection programme. This programme was partly supported by IBPGR, FAO. The MET variety is an open-pollinated flint type. Given ample space, the plants develop three to four tillers with seven to ten ears. This characteristic is genetically determined. The total dry-matter production of the variety planted on 0.1 ha was 500 kg more than of a leading single-cross hybrid. The higher dry-matter production was mainly due to a higher weight of leaves. The nutritive value of the silage made from the variety was compared with that of the single cross. In dry matter, content of total digestible nutrients of the variety was 712 g/kg, for the hybrid 647 g/kg. However content of digestible crude protein and nitrogen-free extract of the variety were lower than of the hybrid. Protein and fat content were similar for the variety and the hybrid. Depending on the growing conditions, the variety is susceptible to lodging and to leaf diseases. A breeding programme to improve agronomic characteristics of the variety is under way.

Descriptors: silage maize, Zea mays L. tillering, prolificacy, digestibility
Relative efficiency of direct and indirect mass selection for biomass yield

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To increase biomass in a long-term experiment, the relative efficiency of 2 procedures of mass selection is being investigated. The study started in 1978 with a specially composed population. The procedures studied are:
- Direct mass selection for top biomass yield when harvesting at the end of the growing season.
- Indirect mass selection for biomass by selecting plants with a top plant height before pollen distribution. These plants are the pollinators because the other plants are emasculated. At the end of the growing season plants with top biomass yield are selected among all plants.

The procedures are applied in the form of grid selection. In 1983, the progress due to a single cycle of selection in the preceding season was determined by comparing the biomass of progenies from selected plants with the biomass of progenies from random plants. The progress amounted to 9% for the direct method and 14% for the indirect method.

Since 1984, the auxiliary criterion for indirect selection is plant volume before pollen distribution, roughly determined as (smallest stem diameter at a height of 50 cm) \( \times \) (largest stem diameter at a height of 50 cm) \( \times \) (plant height), because the genetic variation for plant height seemed to be exhausted.

The level of biomass of the material obtained until 1983 amounts to:

<table>
<thead>
<tr>
<th>Method</th>
<th>Biomass (t/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct method</td>
<td>18.3*</td>
</tr>
<tr>
<td>Indirect method</td>
<td>17.0</td>
</tr>
<tr>
<td>Buras (double-cross grain hybrid)</td>
<td>17.0*</td>
</tr>
<tr>
<td>Splenda (three-way silage hybrid)</td>
<td>19.7</td>
</tr>
</tbody>
</table>

The (non-)significance indicated is in comparison to Splenda.

Descriptors: maize, *Zea mays* L., selection criteria, selection procedures, mass selection
Structure and digestibility of stem cell walls of maize (Zea mays) grown at different temperatures

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2. Department of Field Crops and Grassland Science, Agricultural University, Haarweg 333, 6709 RZ Wageningen, the Netherlands

Zea mays (cv. LG 11) was grown at either 18/12 °C or 30/24 °C day and night, respectively (Struik et al., 1985). Plants were harvested at grain set, which was 93 and 51 days after planting, respectively. Digestibility in vitro of stem cell walls with rumen fluid over 48 h was 70 and 40% for 18/12 and 30/24 °C respectively, at that stage. Stem sections of the 18/12 plants showed thicker cell walls, e.g. more parenchyma wall material and a more intensive histological staining for phenolic compounds (lignin) than of the 30/24 plants. After 24 h incubation in vitro, the outer parenchyma of the 30/24 plants and the central parenchyma of the 18/12 plants had disappeared, and after 48 h all parenchyma from both types had been disappeared. The vascular bundles, sclerenchyma and epidermis could still be identified despite considerable digestion. The warty layer (Engels & Brice, 1985) which adheres to the cell wall and which prevents rumen microflora from digesting the cell wall from the undamaged side of the cell lumen, also occurs in maize. In Zea mays, this layer developed during maturation and drying out of the plant. Tissues without this warty layer digested very rapidly but tissues with this layer showed a very slow rate of digestion. The origin and chemical composition of the warty layer will be investigated. This research has shown that the properties and relative contribution of the cell-walls in the different tissues as affected by temperature have a great impact on cell-wall digestion in ruminants. Varietal differences in digestibility of forage maize will be investigated.

References


Descriptors: maize, Zea mays L., digestibility, cell wall, warty layer, temperature
Response of two genotypically different maize strains in vitro: comparative cytology

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To examine the cytological changes accompanying the various responses of monocotyledons during culture in vitro, immature embryos of two genotypically different maize strains (A188 and A632) were cultured on a medium based on that of Green & Phillips (1975). Before culture, the embryos were selected by scutellum length (between 1 and 3 mm). Initially and after 20 h in culture, embryos were sampled and fixed by standard methods in 4% glutaraldehyde for light microscopy and in 2.5% glutaraldehyde and 1% OsO₄ for electron microscopy. A comparison was made of the scutellum cells of both strains.

At the start of culture, scutellum cells from the A188 line were characterized by a low cytoplasmic density and by medium-sized vacuoles, whereas the A632 cells had denser cytoplasm. The vacuoles were smaller and often contained droplet-like structures. Moreover, extended profiles of plastids were visible in this line. This view was confirmed by electron microscopy, showing more free ribosomes and more RER in A632 cells, while their vacuoles contain round osmiophilic structures.

After culture in vitro for 20 h, the scutellum cells of A188 showed increased vacuolation. The cytoplasm was denser because of a higher concentration of ribosomes. The A632 cells had also increased their content of RER and free ribosomes. Vacuolation, however, had not increased.

The two maize strains differ in cytological features before culture and after 20 h of culture in vitro. Whether these differences are associated with the different responses in vitro of the two lines has still to be further examined.

Reference


Descriptors: maize, Zea mays L., culture in vitro, cell structure
Possibilities of using chlorophyll fluorescence as a rapid non-destructive screening method for cold tolerance

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Part of the light absorbed by green plants is re-emitted as red fluorescence of chlorophyll. After darkness, light induces specific changes in chlorophyll fluorescence, which are related to the functioning of the chloroplast.

Two ways are presented of using induction kinetics of chlorophyll fluorescence in comparing the effects of low temperature stress in vivo. First, one can measure the temperature-dependent changes in the maximum induced fluorescence. In leaf discs of chilling-sensitive cucumber, two break-points were observed: one around 6 °C, indicating the beginning of chilling damage; and one around 18 °C, which may be related to the ability to grow at suboptimal temperature. Secondly, the decrease in the fluorescence rise during exposure to chilling (0 °C) was measured in maize for a cold-sensitive single cross (SH 10) and a cold-tolerant population (SVP). Contrary to cold-tolerant SVP, cold-sensitive SH 10 showed an increase in chlorophyll fluorescence preceding a decrease during the first 8h at 0 °C. The increase can be ascribed to a greater inhibition by chilling of the electron transport in the chloroplast, probably related to sensitivity to cold-induced chlorosis.

Descriptors: maize, *Zea mays* L., *Cucumis sativus* L., cold tolerance, chlorophyll fluorescence
Genetic variability in the rate of leaf appearance in maize during the early stage of growth

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The rate of appearance of young leaves, ligule not emerged, was measured for F1 hybrids in a 20 × 4 top-cross mating design. The number of young leaves was recorded from emergence till the 14-leaf stage. The rate of leaf appearance between two dates was plotted against heat units. We present the pattern of the genetic variance of this trait as measured at various intervals during the early stage of growth. A separate plot was made for each of the four following groups: flint lines crossed with two flint tester lines and two dent tester lines, dent lines crossed with the same pairs of testers.

All the curves were marked by an increase in the genetic variance at the 4-leaf or 5-leaf stage. This phenomenon was related to the transition from the seed-dependent stage to the autotrophic stage. The genetic variance in rate of leaf appearance increased also after the 10-leaf stage.

Major differences were noted between flint material and dent material: at the transition to autotrophic growth, flint testers were responsible for large genetic variance in both flint and dent material, whereas for dent testers, the variance was smaller, reflecting maternal effects; after the 10-leaf stage, no difference was noted between testers, but flint lines showed greater variance.

The pattern of genetic variance in rate of leaf appearance could change over time during the early stage of growth.

Descriptors: maize, Zea mays L., leaf appearance, heat units, genetic variance, maternal effects
Genetic analysis for grain yield of inbred lines of maize selected from different cycles of BSSS synthetic

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The BSSS synthetic was developed in the late 1930s in the United States by G.F. Sprague from 16 inbred lines of different origin resistant to stalk lodging. Abundant literature is available on the agronomic traits of the synthetic per se, and for different versions and cycles. On the other hand, few reports deal with differences in combining ability of inbreds selected from different cycles of selection.

The objective of this study was to measure the combining ability, the components of genetic variance and the heritability of major agronomic traits of inbreds selected from different cycles of the BSSS synthetic: B14 (initial cycle); B73 (C5); B84 (C7). These inbreds were crossed in a diallele without reciprocals with other unrelated inbreds. Sets of parents and F1 hybrids were planted in a randomized block design with four replications at a plant density of 5.86 m². The trial was run in 1983 and 1984. Only results of grain yield are presented here.

The highest average yield in crosses with other unrelated inbreds was given by B84, which was, however, not significantly better than B73. The average yield in crosses of B84 was significantly higher than that of B14. The dominant portion of the total genetic variance as well as heritability was highest in the diallel set with B84 and lowest with B14.

Descriptors: maize, Zea mays L., recurrent selection, grain yield, heritability, genetic variance
Kernel milk-line as a visual maturity indicator in maize (Zea mays L.)

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Water content in kernel and absolute kernel weight at different stages of kernel development were measured in three maize hybrids, ZPSC 42, ZPSC 412 and ZPSC 500/9, in 1983 and 1984. The milk-line represents the border between the solid and liquid kernel contents. Crookston and co-workers in the United States have done extensive research in this field. As the grain matures, the milk-line ‘moves’ from the tip to the basis of the kernel. This process is easy to observe.

Plants were sampled at the following stages: soft-dough (beginning of milk-line in the kernels at the butt of the ear); dented (all kernels with milk-line formed, though not externally visible); half-milk-line (milk-line mid-way down the kernel face), and maturity (indicated by a black layer).

Under normal conditions of growth, the disappearance of the milk-line usually coincides with physiological maturity. As the milk-line is easier to observe than the black layer, it can be used in establishing the stage of kernel maturity. This by no means excludes the use of the black layer. On the contrary, both the black layer and the disappearance of the milk-line should be employed as indicators of kernel maturity in maize.

Descriptors: maize, Zea mays L., maturity, black layer, grain filling
A comparison of hybrids for silage quality

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Breeding high-quality maize for silage with high grain content raises the question of the digestibility of the dry grains at the recommended harvest time for silage, when total plant dry matter content is about 350 g/kg. Therefore, two maize hybrids were grown under drip-irrigation at the Newe Ya’ar Experiment Station in Israel. The hybrids were Halamish, locally the most common forage maize hybrid, and JX180, a hybrid from Jacque Co., United States. Content of ears in total dry matter was 440 and 570 g/kg respectively. The total dry matter yields were 24.38 and 19.25 t/ha for Halamish and JX180, respectively.

The maize hybrids were cut for silage and fed to Friesian and beef-breed young bulls. JX180 was also cut 60 cm above the ground to discard the less nutritious lower part of the stalk. Halamish silage had higher contents of fiber components and ash than JX180. Halamish was not lower in digestibility and metabolizable energy than JX180, whether it was cut high or low above the ground. Moreover, the average daily gain by the Friesian and beef-breed bulls fed on Halamish was not lower than the average gain of bulls fed on JX180 silage, but the Friesian bulls fed on Halamish seemed to consume dry matter at a greater rate. The silage represented 50% of the total diet. By harvest for silage, the high-ear maize hybrid (JX180) had more dry grain than the low-ear maize hybrid (Halamish). Therefore, JX180 silages had higher content of broken and whole kernels in the silage, and gave rise to more grain in faeces. The good performance of bulls with Halamish silage, with less ears in total dry matter, can be related to less grain in the silage and in the faeces of the bulls.

Descriptors: maize, Zea mays L., silage production, ear content, feeding trials
Assessment of yield and quality of silage maize

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In 1982, a 3-year trial with silage maize (5 varieties, 4 harvest-dates) was set up at 3 sites in Switzerland. The aim was to detect sufficiently significant differences between 'normal' and typical 'silage' maize varieties, considering their use as silage maize to give reasons for the establishment of a special breeding and varietal testing program. The varieties under investigation were Protador (FRG) and Eldor (CH), recommended as silage maize, LG 11 (F) as a grain maize variety, and Tau (FRG) and Leader Pau 207 (F) as intermediate forms. The main criteria to test the suitability for silage maize were digestibility of the whole crop as a qualitative attribute and the yield of digestible organic matter as a quantitative character (cellulase method of Jones & Hayward) within a certain range of dry matter content in the whole plant. Results were adjusted to a dry matter content of 520 g/kg in the ear.

The differences in digestibility of the organic matter were about 2.5 % (relative difference 3.5 %) for whole plant and were significant; differences in yield were about 1.5 t/ha (relative difference 11.5 %) and were also significant. A certain compensation effect between quality and productivity was observed. The most productive variety was Eldor with a medium quality; the less productive varieties were Protador and Tau with the poorest quality.

Alongside the investigations of digestibility with the mentioned method, cell-wall constituents were estimated and an Infra-Analyzer was used. For introduction of a silage maize breeding and varietal testing program with thousands of samples to be analysed within a limited time, the Infra-Analyzer method is effective.

Descriptors: silage maize, digestibility of organic matter, yield of digestible organic matter, testing methods
Combining ability of maize (Zea mays L.) inbred lines in different generations of inbreeding

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Six inbred lines of maize in different generations of inbreeding (S2, S5, S8) were selected to study their combining ability for major agronomic traits. The analysis of combining ability was done according to Griffing (1956), Method 2, Mathematical Model I.

The ratio of general to specific combining ability (GCA/SCA) was lower than 1 for the following traits: grain yield per plant, individual kernel weight, ear length and plant height. This ratio indicates dominance of non-additive gene action in the expression of these traits.

For number of kernel rows, GCA/SCA was greater than 1, i.e. additive gene action was dominant for the expression of this trait.

The test results showed that lines from earlier generations of inbreeding (S2) usually gave lower values of SCA, whereas lines from later generations of inbreeding (S5, S8) showed pronounced heterosis.

Descriptors: maize, Zea mays L., combining ability, heterosis
Performance of early versions of hybrids developed from late elite maize inbred lines

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Early versions of maize inbred lines were developed from their original late elite versions B73 (American) and ZP 70/9 and ZP 307/17 (Yugoslav). Inbred S-61 was used as source of earliness for all three inbreds. Tg 348 was an additional source of earliness for B 73. Trial results, averaged for three years, indicated that the best early versions of inbred lines had a shorter time from emergence to tasselling, and lower plant and ear height than their original versions. Number of ear rows and ear length were comparable to the late elite lines.

In 1984, trials were laid out at six sites with two hybrids of FAO maturity group 500, developed by crossing early versions of B 73 with Mo 17. The hybrids gave a grain yield higher than controls NS 555 and NS 444 by 14.4% and 15.9%, respectively. An early version of ZP 307/17 crossed with B 73 yielded more than the above controls by 17.7% on average. Kernel moisture content at harvest of new hybrids was the same as for controls.

Descriptors: maize, Zea mays L., earliness
Selection for earliness in Corn Belt maize populations

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Two methods of selection for early flowering were compared for three Corn Belt maize populations: BSSS (HT), Lancaster and BS10. Both methods consist of two generations per cycle. In Generation 1 of Method A (G1A), the 100 earliest flowering plants out of 1000 were selfed to create S1 families. In Generation 2 (G2A), the three earliest plants out of 20 within each of the 30 earliest S1 families out of 80-100 were selected and hand-pollinated with bulk pollen from the selected plants. Before starting Method B, each population was crossed with a donor for earliness. In Generation 1 (G1B), the 100 earliest plants out of 1000 from the cross were bulk-pollinated. In generation 2 (G2B), the 100 earliest plants out of 1000 were back-crossed with bulk pollen collected from the selected plants in the simultaneous G2A generation.

Cycles 1A and 2A from Method A, and 0B, 1B and 2B from Method B were compared in two environments. Populations per se, intercross populations and topcrosses of advanced selection cycles were all significantly earlier flowering, had a lower moisture content of grain at harvest and were more lodging-resistant than previous cycles. No significant differences were found for grain yield and early vigour.

Descriptors: maize, Zea mays L., recurrent selection, earliness, maturity, lodging resistance
Testing maize for silage and corn-cob mix at Weibullsholm 1974-1984

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In the 1950s, breeding and testing of maize were extensive in Sweden. A compilation of the results was given in the book 'Studies of hybrid maize for silage' published in 1959. The variety Prior from CIV in the Netherlands with an FAO maturity rating of 240 became the most popular variety after that time. 'Prior' was included in the Swedish Variety List in 1963.

When I began research on forage crops at Weibullsholm in 1973, maize had become popular in Germany; in Denmark much work was being done on testing and cropping of maize. Consequently, the official varietal trials started again in Sweden in 1974.

The seed rate recommended in our year-book at that time was 65-85 kg/ha for 'green fodder maize' (plant density 20-30 m$^2$). This rate has gradually diminished, first to 50 kg/ha, then to 40 kg/ha. Every year since 1974, we have tested 50-75 market varieties from different institutes and countries. Our most intensive contacts have been with breeding stations such as Limagrain, RAGT, KWS, Van der Have and Zelder but we have also, for instance, tested varieties from Ciba Geigy, institutes in Canada, Nordsaat, Cargill and INRA. In the yield trials, we worked with three groups of material:

- Silage, middle early – middle late, FAO 230-260, with LG11 for standard
- Silage, early – middle late, FAO 200-230, with Boree for standard
- Corn-cob mix, early, FAO 180-210, with Boree for standard.

For each group of material, about 10 new and all commercial varieties were tested each year in observation trials. The yield trials were always done with two plant densities i.e. 7-8 and 9-10 m$^2$. The total dry-matter yield is, on average, 13500 kg/ha for LG11 and 13000 kg/ha for Boree with an ear dry-matter content of 430 and 460 g/kg, respectively and an ear content of 450 and 480 g/kg in total dry matter, respectively.

In the 'Official Swedish List of Cultivars 1984-85' 9 maize varieties were included, of which we represent in Sweden Edo (1978), LG11 (1979), Galion (1982) and Boree (1982).

Descriptors: silage maize, Zea mays L., corn-cob mix, varietal testing, Sweden
Performance of early maize hybrids for grain and silage grown as second crop in northwestern Croatia

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Maize Hybrids belonging to maturity groups FAO 100-400 were grown as second crop after canning or forage peas, winter barley and winter oilseed rape in northwestern Croatia at Zagreb – Maksimir for four years (1981-1984). The following results were obtained.

After peas, maize was planted on 14 June to 2 July. FAO 100 hybrids matured to content of grain moisture 350-400 g/kg and gave grain yields of 3.5 to 4.5 t/ha; FAO 200 hybrids gave similar grain moisture at harvest only in ‘warm’ years (1982 and 1983); in ‘normal’ (1981) and ‘cold’ years (1984), they gave dry-matter (DM) yields of whole plants (DMY) of 7-8 t/ha with content of dry matter more than 250 g/kg; FAO 300 and 400 hybrids matured for fodder ensiling and gave DMY 7.7 to 9.0 t/ha with good DM content (260 to 350 g/kg) in ‘normal’ and ‘warm’ years, but lower DMY (5.7 to 7.1 t/ha) and DM content (200-240 g/kg) in ‘cold’ years.

After winter barley, maize was planted on 28 June to 12 July. FAO 100 hybrids matured to high moisture content (380-420 g/kg) for grain in ‘warm’ years and for silage in ‘normal’ and ‘cold’ years and gave DMY of 6.3 to 7.0 t/ha with DM content 280 to 290 g/kg in ‘normal’ and 200 g/kg in ‘cold’ years. FAO 200 hybrids were too moist for grain production but could be used for good silage (DMY 7 to 8 t/ha) in ‘normal’ and ‘warm’ years; FAO 300 hybrids gave high DMY only in ‘warm’ years.

After winter oilseed rape, maize was planted from 28 June until 17 July. Hybrids from the maturity groups 100 and 200 gave DMY 7.6 to 8.9 t/ha and high DM content (260 to 320 g/kg) only in ‘warm’ years, but in other years DMY was 4.2-6.0 t/ha with low DM content (186-227 g/kg).

Descriptors: maize, Zea mays L., second crop, silage production, grain production, Croatia
Effect of temperature on accumulation and partitioning of dry matter during early phases of development in maize

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Total and component plant dry weights were determined at three leaf stages (4-, 8- and 12-leaf stage for two maize hybrids, PAG SX111 and Pioneer 3851, grown at four mean temperatures (15, 19, 23 and 27 °C), at either constant day-night or 12 °C day-night differential temperature regimes. In addition, CO₂ exchange rates at 650 and 2500 μmol m⁻² s⁻¹ (the former was the prevalent radiant flux density during the day-time growing conditions) and photosynthetic efficiency were measured at the 12-leaf stage.

In general, there were only minor differences between constant and day/night differential temperature regimes for the parameters measured. All photosynthetic parameters measured showed a significant linear response to an increase in temperature; photosynthetic rate of PAG SX111 was slightly higher than that of Pioneer 3851 at 650 μmol m⁻² s⁻¹, but hybrids did not differ for the other two photosynthetic parameters. In contrast, total plant dry matter and leaf area index at the 12-leaf stage were highest at 19 °C and 19 to 23 °C, respectively. Both root partitioning coefficient and specific leaf weight declined rapidly with increasing temperature and attained a minimum at 23 °C. Dry weight and leaf area index of Pioneer 3851 were significantly higher than those of PAG SX111 throughout the period measured, and the only parameter measured in this study to which this difference could be attributed, was a root partitioning coefficient about 8% higher for PAG SX111.

Descriptors: maize, Zea mays L., dry-matter accumulation, dry-matter partitioning, temperature
Study on the possibilities of intersubspecies hybridization for improvement of silage maize

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From 1979 to 1984, a breeding programme was established to develop special maize hybrids for silage. Using sweet corn Zea mays saccharata Sturt. and first-cycle inbred lines with 50% germplasm of pop and dent corn, as source material, six kinds of hybrid were developed, containing 50, 33, 25 or 17% popcorn and 50 and 33% sweet corn. The hybrids were tested for grain yield and the protein content of the biomass was analysed.

A negative correlation was found between yield and proportion of germplasm from pop and sweet corn in the hybrids. Reduction of the proportion of popcorn germplasm from 50 to 33%, and from 25 to 17% resulted in a dry-matter increase of 7.3 and 13.8%, respectively. In the hybrids, a positive correlation was found between protein content and the proportion of popcorn and sweet corn. Increase of popcorn and sweet corn germplasm from 17 to 50%, and for sweet corn from 33 to 50% resulted in an increase of the protein content from 9.4 to 13.5% and 5.9%, respectively. On the basis of our results, introduction of pop and sweet corn germplasm into hybrids could increase protein content in the dry matter to a good value.

Descriptors: maize, Zea mays L., silage production, wide crosses, protein content
Breeding of maize hybrids for whole-crop silage in Japan

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Maize is one of the major forage crops used as whole-crop silage for dairy cows and beef cattle all over Japan. Breeding objectives are high nutritional yield and quality, resistance to lodging by typhoon, and to economically important diseases. Cold tolerance is essential in northern Japan. Efforts are concentrated on developing new inbred lines by combining domestic Caribbean flint and Northern flint germplasm with foreign high-yielding material. Evaluation and selection criteria for hybrids suitable for central and southern Japan are the following: TDN (total digestable nutrients) yield, TDN yield/time of growth, ear ratio, proportion lodging, southern leaf-blight disease score, incidence of banded leaf and sheath disease, and of smut disease. Each hybrid is ranked from A to D for each of these criteria, subsequently an individual hybrid score calculated by giving two points to Rank A, one point to Rank B, and zero to Rank C, and totalling the points given. If a certain hybrid is given Rank D in a single character, the hybrid is eliminated from selection because of its inferiority, regardless of its total score.

Descriptors: silage maize, *Zea mays* L., breeding, Japan, selection criteria, nutritional yield, lodging resistance, disease resistance, domestic germplasm
Utilization of local Spanish maize populations

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The introduction of hybrids from the United States in all Europe rapidly ousted the adapted local varieties. In Spain, however, more than 80,000 ha is still planted with local populations. In 1958, we made our first collection of such populations, which were subsequently divided in 65 local groups. Since 1981, a second collection has been made by the Alcalá (Madrid) Station. At the moment, we are trying to classify the collected material by multivariate analysis. This collection now consists of 1254 samples.

To investigate the potential use of the local groups of the first Spanish collection, 22 late and medium groups were crossed with BS-13 and a Lancaster composite from Iowa State University. The three best crosses were with BS-13 and their yield was similar to B73 \(\times\) Mol7. A 9 \(\times\) 9 diallele between seven Spanish groups and two American stocks also showed that BS-13 had the best combining ability, followed by Blanco and Andaluz. The best cross was Andaluz \(\times\) BS-13.

In 1981, we started to use a recurrent selection method based on the performance of test crosses of S1 lines with B73 and on the performance of S2 lines per se. The average yields of test crosses of the 20% selected lines from the local groups Andaluz sin 6 (A \(\times\) C) C1, Roteno C3 and Hembrilla C2 were similar to B73 \(\times\) Mol7.

Phenotypic recurrent selection for number of ears per plant (A), number of kernel rows per ear (B) and ear length (C), all yield components, was made in Andaluz, Fino, Rastrojero and Tremesino local groups. The selection cycles C4 and C8 of each group were crossed with BS-13 and Lancaster. The best-yielding combinations were Andaluz AC8 and Tremesino AC8 crossed with BS13.

Half-sib selection was made in 10 local groups. Good results were obtained with the Onate group.

Descriptors: maize, Zea mays L., Spanish local varieties, utilization
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