The effects of low temperature on seedling growth of maize genotypes

P. Miedema, J. Post and P.J. Groot

Foundation for Agricultural Plant Breeding, Wageningen

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Abstract

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This report describes investigations of what selection criteria are required for breeding cold-tolerant maize. The effects of low temperatures on seedling growth were studied from germination until appearance of the sixth leaf. Ten single-cross hybrids of flint and dent maize were tested for cold tolerance traits under field, greenhouse and growth-chamber conditions; some experiments were carried out with varieties and a group of exotic accessions. Low temperatures before emergence caused seed and seedling mortality, chilling injury, retarded emergence and reduced seedling vigour. After emergence, low temperatures caused chlorosis, retarded leaf extension and shoot dry matter accumulation. Genotypic variation was recorded for the low-temperature responses but most of them were statistically not correlated with each other. This suggests that different genetic and physiological characteristics are involved. Resistance to chlorosis and rapid leaf extension at low temperature are considered major selection criteria for the improvement of low-temperature adaptation.

Free discriptors: *Zea mays*, maize, genotypic variation, cold tolerance, low temperature, seedling growth, chilling injury, chlorosis, germination, emergence, shoot morphology, leaf extension, shoot dry matter accumulation.

Contents

Abbreviations and symbols

LA	leaf area
RGR	relative growth rate
RLAGR	relative growth rate of leaf area
RSGR	relative growth rate of shoot
SH	single-cross hybrid
p	path coefficient
r	simple correlation coefficient
R^2	coefficient of multiple determination
$\bar{x}$	sample mean
s.d.	standard deviation
$s_{\bar{x}}$	standard deviation of the sample mean
*	statistically significant at $P \leqslant 0.05$
**	statistically significant at $P \leqslant 0.01$

1 General

1.1 Introduction

During the last 15 to 20 years the maize-growing area in North-Western Europe has been increased considerably. A major part of this increase is the result of the introduction of silage maize in northern regions that are too cool for grain maize (Bunting, 1978, 1980). In the Netherlands the area of silage maize increased from 4 000 ha, in 1969, to 196 000 ha, in 1986. Chemical weed control and mechanization of seeding and harvesting were major factors that made the expansion possible. Early-maturing grain-maize varieties developed for the traditional maize areas in France and Germany were used for silage in cooler regions. Later on new varieties were developed that outyielded the original grain-maize varieties (te Velde, 1984).

A major growth-limiting factor in northern areas is the temperature at the beginning of the season. Low temperatures may result in several types of damage, such as seed rot, frost injury and chlorosis. Low temperatures above the injury threshold will retard emergence and vegetative growth. The grain maize varieties already mentioned have some degree of cold tolerance, but further improvements are possible (Dolstra & Miedema, 1986). Adaptation to low temperatures has been an important objective in the breeding of silage maize for northern areas. Screening for this adaptation is usually done under cool field conditions, on such selection criterea as percentage of emerging seedlings, rate of emergence, resistance to chlorosis and vegetative plant growth. The question arises: how effective is this screening? First, field screening is subject to fluctuations in temperature; with a warm season no screening is worthwhile. Second, little is known about interrelationships between various cold-tolerance traits. Third, little quantitative data are available about the effect of cold tolerance on initial growth in terms of dry matter and leaf area. Initial growth is an important characteristic in maize breeding, as shown by te Velde (1986), who reported a good correlation (r = 0.78) between initial growth and final dry matter yield in 12 silage maize varieties tested over five years. More information on selection criteria may help make breeding and screening more effective.

We started investigations on cold tolerance in maize in 1971, with special emphasis on physiological aspects of genotypic differences. The ultimate objective was to find plant characteristics that limit growth at low temperature and the appropriate screening techniques to be used. This agricultural research report contains the experimental result of our investigations. A review of the literature, as well as some of the main results of our investigations, has been published by Miedema (1982). Other reviews on

the effects of low temperature on maize have been published by Stamp (1984c) and Crèvecoeur & Ledent (1984).

1.2 Plant material

Many experiments have been carried out with a group of 10 single-cross hybrids (Table 1) that differ in cold tolerance under field conditions; the group comprised five flint and five dent hybrids. Some experiments were carried out with commercial varieties (three-way or double-cross flint x dent hybrids) more or less adapted to the climate of North-Western Europe. Finally, a group of exotic accessions comprising various landraces and CIMMYT gene-pool populations was tested. Seed of the commercially available varieties was supplied by seed companies; seed of the other materials was produced in a greenhouse.

1.3 Methods

The effects of temperature were studied from germination until emergence and from emergence until the tip of the 6th leaf appeared (6th-leaf stage). Stages of plant development after emergence were indicated by the number of *visible* leaves and not by the number of fully grown leaves, which is usual (e.g. Hanway, 1963; Groot et al., 1986). For comparison of both systems: in the 4th-leaf stage the 1st leaf is full-grown (collar visible); in the 6th-leaf stage the 3rd leaf is full-grown.

Five types of growth conditions were used:

- Dark rooms with temperature conditioning; seeds or seedlings in soil or moist perlite (e.g. Sections 3.2 and 4.2).
- Growth cabinets with artificial light (Section 5.2); plants in pots with potting compost.
- Glasshouse experiments with (Sections 6.3 and 6.4) or without (Section 5.3) temperature conditioning; plants in pots with potting compost.
- Outdoor experiments with potted plants (e.g. Sections 5.3 and 7.2).
- Field experiments (Section 2.2).

Detailed descriptions of these conditions are presented in the sections indicated.

The effects of temperature were assessed by visual observation of damage and by investigating plant growth as extension growth or shoot dry-matter accumulation. Extension growth included shoot elongation just after emergence and leaf elongation, estimated by periodical measurements with a ruler of the distance between the soil level and the tip of coleoptile or first leaf, or the tip of the leaf involved, usually the 3rd leaf. Shoot dry weight was estimated by oven drying (at 105 °C for 16 – 24 h) of shoots cut off at the coleoptilar node. Relative growth rate of the shoot (RSGR) was calculated using methods common to growth analysis (e.g. Radford, 1967). In some cases leaf area (LA) and its relative growth rate (RLAGR) were estimated (see Sections 5.2.2 and 5.5.2).

Environmental variation is usually large in seedlings of maize. Therefore, several

plants (20 – 50) were investigated per treatment and per genotype. Mean values and standard deviation of the mean ($\bar{x} \pm s_{\bar{x}}$) were calculated. For several traits, correlations (r) have been calculated. In some cases analysis of variance was done. Path-coefficient analysis was carried out for the major components of cold tolerance in the group of single-cross hybrids (Chapter 8).

1.4 Scope of the investigations

The experimental work focused on adaptation of maize to the cool maritime climate of the Netherlands, characterized by slow increase of temperature in spring and summer. Diurnal fluctuations are compartively small; for the months May-September the difference between maximum and minimum is on average around 10 °C. In the Netherlands, maize is sown at the end of April and harvested in October. Temperature is a limiting factor mainly during early vegetative growth. Experiments, therefore, were restricted to the seedling stage.

The experiments are described in Chapters 2 to 7; those chapters can be read separately. Chapter 2 describes the effects of cool conditions, which will be met by early sowing, on percentage and rate of emergence, chlorosis and shoot dry weight in the 6th-leaf stage in the 10 single-cross hybrids. The data of this field experiment are a basis for further analysis of effects of low temperature under controlled conditions, described in Chapters 3, 4, 5 and 6.

Chapter 3 describes the effect of temperature (6 – 40 °C) on the rate of germination and shoot extension before emergence in cv. Fronica, and the rate of germination and emergence at 12 °C in the 10 single-cross hybrids and a group of 11 exotics.

Chapter 4 describes various types of low temperature damage in imbibed seeds and germinated seedlings of the 10 single-cross hybrids and other genotypes.

Chapter 5 presents a detailed description of seedling growth after emergence of 9 (of the 10) single-cross hybrids under different environmental conditions in growth cabinets, a greenhouse and outdoors.

Chapter 6 describes low temperature damage after emergence in some varieties and some of the single-cross hybrids.

In Chapter 7, experiments with a group of various exotics to investigate whether the main results obtained from the 10 flint and dent single-cross hybrids also apply to other material are described.

Chapter 8 describes path-coefficient analysis of data from the single-cross hybrids.

Chapter 9 contains a discussion of the main results, and some new data from literature not included in the review paper by Miedema (1982).

2 The effects of early sowing on emergence and seedling growth of ten single-cross hybrids

2.1 Introduction

A group of ten single-cross hybrids were used in many experiments of this study. Single-cross hybrids were chosen because they are genetically (more) uniform than varieties and they are more vigorous than inbred lines. The plant material consisted of genotypes that differed in response to low temperature under field conditions.

In the Netherlands, the period between 20 April and 1 May has been recommended as the most suitable for sowing of maize (Becker, 1976). Sowing before 20 April increases the risk of low-temperature damage during germination or damage by night frost. Sowing after 1 May may facilitate emergence and seedling growth but final yield is often reduced by the shorter growing season. Average monthly temperatures (°C) in the Netherlands are:

Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
2.0	2.3	4.8	8.0	12.1	15.2	16.6	16.4	14.0	10.3	5.8	3.2

These data are averages of hourly recordings at the meteorological station KNMI, De Bilt, in the middle of the country.

The experimental material was planted on six dates in the period 19 March tot 19 June; these six trials are designated by the symbols S1, S2. . .S6. The aim of the experiments described in this chapter was to determine the effects of low temperature on the ten single-cross hybrids. Date of emergence, percentage of emerging plants and dry weight of shoots at the 6th-leaf stage were determined; in the early sowings, resistance to chlorosis was assessed. Relationships between those characteristics were studied to trace common factors in the response to low temperature.

2.2 Material and methods

The ten genotypes and their characteristics are listed in Table 1. The inbred lines were provided by the breeding company Zelder BV, Ottersum, the Netherlands. The figures for cold tolerance of the single-cross hybrids were given by Ir. H.J.H. Weijs of Zelder BV. They are based on visual observations of seedling vigour and chlorosis under field conditions over a period of several years.

Seeds of the single-cross hybrids were produced under favourable conditions in a greenhouse in 1974. The seeds were hand-threshed, dried, and stored at 2 °C and

Table 1. Characteristics of the 10 single-cross hybrids used in experiments. Cold tolerance is indicated on a 1 – 9 scale: 1 is the most sensitive and 9 the most tolerant to low temperatures under field conditions.

Genotype	Kernel type	Cold tolerance	Kernel weight (mg)	Parental lines	Origin
SH1	flint	9	379	A × B	Netherlands
SH2	flint	8	256	C × B	Spain, Netherlands
SH3	flint	6	251	F7 × F2	France
SH4	flint	6	250	D × E	Spain, various
SH5	flint	5	299	G × H	various
SH6	dent	4	320	WH × WJ	USA
SH7	dent	3	311	W182E × W33	USA
SH8	dent	2	256	W37 × W79A	USA
SH9	dent	1	272	W153R × W374B	USA
SH10	dent	1	284	W153R × W182B	USA

Table 2. Dates of sowing and harvest, duration of the periods from sowing to emergence, and emergence to harvest, and average air temperatures during those periods. The duration of the periods is based on the mean dates of emergence (see Table 4).

Trial	Sowing date	Harvest date	Period sowing to emergence		Period emergence to harvest	
			time (days)	temperature (°C)	time (days)	temperature (°C)
S1	19 March	11 June	53	6.8	31	12.0
S2	2 April	11 June	37	8.1	33	11.1
S3	16 April	11 June	23	10.4	34	11.1
S4	30 April	13 June	18	11.4	26	13.0
S5	14 May	17 June	14	12.0	20	14.8
S6	18 June	11 July	7	18.2	18	15.2

30 % relative humidity. Seed weight was determined after exposure for a few hours to ambient laboratory conditions. Moisture content was not determined but it will have been similar for the seed lots. Seeds were dressed with a mixture of fungicide and insecticides that contained 250 g thiram, 100 g lindane and 250 g methiocarb per kilogram of mixture.

Six sowing dates, in 1975, were used (Table 2). The experiment incorporated a randomized block design. On each sowing date, eight replicates (rows) of five seeds per genotype were planted. Plant distance was 10 cm in the row and 50 cm between the rows. The experiment was carried out on a sandy soil. Soil moisture was sufficient

during the experimental period. On some nights all plots were covered with transparant plastic sheeting to prevent damage by night frost.

Air temperature at 10 cm above ground level (+ 10 cm) and soil temperature at 5 cm below level (− 5 cm) were recorded with thermographs. Average day temperatures were calculated; average temperatures of weekly periods are presented in Figure 1.

Dates of emergence were estimated for individual plants by daily observations. The numbers of emerged plants of sowing dates S1, S2 and S3 were determined on 28 May, and of S4, S5 and S6 at harvest time. On 18 May, chlorosis and intermediate plant size of S3 material were assessed by visual observation. Shoots were harvested at about the 6th-leaf stage (6th leaf just visible). Harvest dates are given in Table 2. Shoots were cut off at soil level (in all other experiments at the coleoptilar node); shoot dry weight was determined for individual plants. Plants that had not emerged and clearly abnormal plants that were damaged or had emerged extremely late were omitted in the calculation of date of emergence and shoot dry weight (see Table 3 number of 'normal' plants).

An additional experiment was carried out in which plants of the 10 single-cross hybrids were grown in pots (diameter 16 cm) with potting compost. Twenty seeds were used from each genotype. After sowing, the pots were kept in a greenhouse at 18 – 23 °C for 4 days. The pots were put outside on 5 May. Date of emergence was recorded. Shoots were harvested on 9 June, when the plants were in the 6th- or 7th-leaf stage.

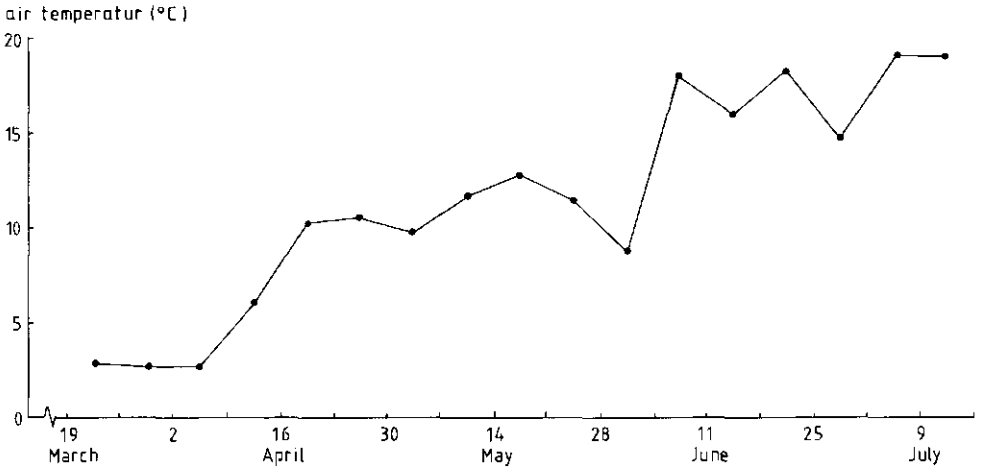

Figure 1. Average weekly air temperature during the experimental period 19 March-11 July 1975.

Table 3. Effect of sowing date on the numbers of emerged plants of 10 single-cross hybrids. For S1 and S2 numbers of emerged plants plus germinated seeds are also given. Numbers of 'normal' plants (see Section 2.2) used to calculate data of Tables 4 and 5 are given. At each sowing date 40 seeds of each genotype were planted. Means within the sowing-date groups followed by the same letter are not significantly different at $P \leqslant 0.05$; arc sine transformation was performed on percentage emerged plants in S1, S2 and S3.

Genotype	Number of emerged plants for each sowing						Emerged plants plus germinated seeds		Number of 'normal' plants for each sowing					
	S1 19 March	S2 2 April	S3 16 April	S4 30 April	S5 14 May	S6 18 June	S1	S2	S1	S2	S3	S4	S5	S6
SH1	19 bc	30 bc	38 a	38	40	38	34	40	18	29	32	33	40	36
SH2	36 e	36 cde	39 ab	38	39	40	36	39	33	33	38	37	38	40
SH3	24 bc	25 b	40 b	38	40	40	27	27	20	23	38	35	38	40
SH4	17 b	24 b	37 a	37	39	40	27	31	14	20	35	33	35	39
SH5	8 a	11 a	36 a	40	38	40	9	14	7	11	32	39	33	40
SH6	25 bc	30 bc	40 b	39	39	39	31	39	21	28	37	38	37	38
SH7	5 a	11 a	39 ab	37	39	40	21	27	5	9	34	33	38	38
SH8	27 cd	33 cde	38 a	40	38	40	38	40	23	31	37	39	38	40
SH9	33 de	36 cde	39 ab	40	40	39	35	38	28	35	38	37	39	39
SH10	20 bc	38 e	39 ab	40	40	39	31	40	17	32	38	39	40	39
Mean	21.4	27.4	38.5	38.7	39.2	39.5	28.9	33.5						

2.3 Results

The air temperature during the experimental period is depicted in Figure 1. Average soil temperatures at −5 cm were similar to air temperature, but daily maxima were 1 – 2 °C lower and daily minima 1 – 3 °C higher in the soil than in the air. Temperature was very low during the first four weeks. During this period, daily maxima of soil temperature did not exceed 11 °C. Mean air temperatures during the periods from sowing to emergence and from emergence to harvest are given in Table 2.

The effects of sowing date on the number of emerged plants, time to emergence and shoot dry weight are presented in Tables 3, 4 and 5, respectively. Shoots of S1, S2 and S3 were harvested on the same day, although plant development of S1 lagged behind S3. On 28 May, seeds of S1 and S2 that had not emerged were dug up. Of the whole group of 312 seeds, 12 % were not found, 44 % showed no signs of germination, 29 % had shoots < 4 cm and 15 % shoots > 4 cm. The smaller shoots were mostly dead and decayed. The longer shoots were alive but had not emerged because of their abnormal morphology: the leaves had grown through the coleoptile and the shoots were distorted and not able to grow in a vertical direction. Some of those seedlings eventually emerged with a folded first leaf. This disoriented growth was mainly found in genotypes SH1, SH4, SH6, SH7 and SH8. The total number of emerged plus germinated plants in S1

Table 4. Effect of sowing date on time from sowing to emergence of 10 single-cross hybrids. Means in column followed by the same letter are not significantly different at $P \leqslant 0.05$.

Genotype	Time from sowing to emergence (days)						Emergence time ratio S3/S6
	S1 19 March	S2 2 April	S3 16 April	S4 30 April	S5 14 May	S6 18 June	
SH1	56.6 de	38.8 bcd	25.5 e	19.2 e	15.4 e	8.7 d	2.93
SH2	48.5 a	32.4 a	21.1 a	16.9 ab	12.9 a	6.7 ab	3.15
SH3	52.9 bc	40.0 cd	23.5 d	17.8 cd	14.0 cde	6.6 ab	3.56
SH4	56.7 dc	37.2 bc	22.9 bcd	18.4 d	14.2 de	7.9 c	2.90
SH5	58.7 e	40.4 d	23.2 cd	17.9 cd	14.2 de	6.7 ab	3.46
SH6	52.3 bc	36.0 b	22.2 abc	17.6 c	13.8 bcde	7.2 b	3.08
SH7	52.4 bc	37.9 bcd	21.5 a	16.9 ab	13.2 abc	7.1 ab	3.03
SH8	51.0 b	37.1 bc	21.8 ab	16.8 a	13.1 ab	6.4 a	3.41
SH9	54.7 cd	36.2 bc	22.4 abcd	17.6 c	13.2 abc	6.6 ab	3.39
SH10	50.3 b	33.8 a	21.2 a	17.5 bc	13.5 abcd	6.7 ab	3.16
Mean	53.4	37.0	22.5	17.7	13.8	7.1	3.21
s.d. of the mean	1.02	0.80	0.42	0.23	0.24	0.23	0.07
Mean date of emergence	11 May	9 May	8 May	18 May	28 May	25 June	

Table 5. Effect of sowing date on shoot dry weight and shoot dry weight ratios of 10 single-cross hybrids harvested at about their 6th-leaf stage. Plant numbers are given in Table 3. Dry weight values in column followed by the same letter are not significantly different at $P \leqslant 0.05$.

Geno-type	Shoot dry weight (mg/plant)						Shoot dry weight ratio	
	S1 19 March	S2 2 April	S3 16 April	S4 30 April	S5 14 May	S6 18 June	½ (S1 + S2)/S3	S3/S6
SH1	300 bc	390 c	468 d	435 d	406 d	349 ab	0.74	1.34
SH2	389 d	499 d	517 e	412 d	401 cd	429 bc	0.86	1.21
SH3	260 b	298 b	391 c	346 bc	392 cd	434 bc	0.71	0.90
SH4	172 a	301 b	383 c	313 ab	352 bc	290 a	0.62	1.32
SH5	256 b	299 b	387 c	346 bc	389 cd	354 ab	0.72	1.09
SH6	321 bc	377 c	390 c	374 c	415 d	473 c	0.89	0.82
SH7	267 bc	291 b	312 b	288 a	319 b	356 ab	0.90	0.88
SH8	342 c	327 b	372 c	354 bc	336 b	395 bc	0.90	0.94
SH9	173 a	224 a	268 a	276 a	272 a	360 ab	0.74	0.74
SH10	259 b	278 b	328 b	311 ab	323 b	403 bc	0.82	0.81
Mean	274	328	382	346	360	384	0.79	1.00
Coefficient of variation	25.0	23.3	18.8	14.8	13.1	13.8	12.3	22.0

and S2 is given in Table 3. Because it was often difficult to distinguish between seeds that had or had not germinated, these data are only indicative.

Assessments of chlorosis resistance and intermediate plant size of S3 material on 18 May are given in Table 6. Wilcoxon's rank sum test showed that the group of flints, SH1 – SH5 had a significantly ($P \leqslant 0.005$) higher chlorosis resistance and smaller intermediate plant size than the dents, SH6 – SH10. Chlorosis in S1 and S2 was similar to that in S3. Plants of S4 were less chlorotic than those of S3. Plants largely recovered from chlorosis when temperature rose (Figure 1). No chlorosis was found in plants of S5 and S6.

Percentage of emerged plants of S1, time to emergence of S3, shoot dry weight of S3 and shoot dry weight ratio S3/S6 were considered to be the main characteristics in which low temperature response was expressed. Correlation coefficients between those characteristics and other characteristics of the genotypes are given in Table 7.

Pot experiment. Shoot length (mesocotyl plus coleoptile) was about 1 cm when the pots were transferred from the greenhouse to the field. Date of emergence and data of shoot dry weight are given in Table 8. The average date of emergence was 9 May, so that shoot growth after emergence coincided with that of S3. Light conditions and air temperature were the same as for S3, but on clear days soil temperature in the pots was about 2 °C higher than soil temperature in the field.

Table 6. Resistance to chlorosis and intermediate plant size of 10 single-cross hybrids sown on 16 April (S3) estimated on 18 May. The plants were in the 3rd-leaf stage. Assessments were made per replicate of five plants, using a 1 – 9 scale: 1 is most yellow and the smallest size; 9 is most green and the largest size.

Genotype	Resistance to chlorosis ($\bar{x} \pm s_{\bar{x}}$)	Intermediate plant size ($\bar{x} \pm s_{\bar{x}}$)
SH1	7.2 ± 0.31	3.1 ± 0.69
SH2	8.1 ± 0.35	5.4 ± 0.18
SH3	6.2 ± 0.16	3.6 ± 0.46
SH4	8.1 ± 0.23	1.5 ± 0.19
SH5	8.2 ± 0.16	2.4 ± 0.37
SH6	1.1 ± 0.12	8.9 ± 0.12
SH7	1.8 ± 0.16	8.1 ± 0.12
SH8	4.8 ± 0.16	6.6 ± 0.18
SH9	3.4 ± 0.16	5.9 ± 0.69
SH10	3.6 ± 0.26	7.1 ± 0.12

2.4 Discussion

Average temperatures during the growth period were higher for later sowing dates (Figure 1 and Table 2). Effects of early sowing are considered, therefore, effects of low temperature. Effects will be distinguished as occurring before and after emergence. Before emergence, average air temperatures ranged from 6.8 °C in S1 to 18.2 °C in S6; after emergence they ranged from 11.1 °C in S3 tot 15.2 °C in S6. The data of the S3 trial will be used as standard for the effects of low temperature because the temperature conditions during the growth period of this trial are representative for a cool spring in the Netherlands. We will first discuss overall effects of low temperature, i.e. effects expressed in the mean data of the ten genotypes, and then the differential response of the genotypes.

Early sowing reduced the number of emerged plants (Table 3). In S1 and S2 the proportion of emerged plants were 54 % and 68 %, respectively, and in S3 and later sowings 96 – 99 %. Most of the non-emerged plants of S1 and S2 died during or after germination, probably because of fungi or physiological damage. Some seedlings were alive but did not emerge because shoot growth was disoriented, resulting in distorted seedlings below the soil surface. The distorted seedlings were similar to those found by Buckle & Grant (1974), which were caused by large diurnal temperature fluctuations and low night temperatures. There, too, disoriented growth occurred when leaves grew through the coleoptile before emergence.

Time from sowing to emergence (Table 4) decreased from 53.4 days in S1 to 7.1 days in S6 as a result of increase of temperature. Early sowing may delay the date of

emergence, since the average date of emergence of S1 lagged three days behind S3. This delay is attributed to the adverse effects of the first four weeks of low temperatures in S1.

Early sowing also influenced shoot dry-matter accumulation (Table 5). Plants of S1, S2 and S3 were harvested at the same date. Within this group of sowing dates, shoot dry weight decreased with earlier sowing. The differences between S1 and S2 may be caused partly by the earlier emergence of S2 seeds. The main cause of the lower yield of the earlier sowings seems to be some adverse after-effect of the long period of low temperature between sowing and emergence. A quantitative measure of this after-effect is the ratio of the average shoot dry weights in S1 and S2, and shoot dry weight in S3, expressed as ½(S1 + S2)/S3. The range of the value of this ratio shows that the after-effects due to genetic variation were rather small. Further experiments on the after-effects of low temperature before emergence and presented in Section 5.3. The average data of shoot dry weight of S4, S5 and S6 cannot be compared with each other because the growth periods were different.

Chlorosis was observed in S1, S2, S3 and to some extent in S4, but not in S5 en S6. This chlorosis was due to low temperatures during the first weeks after emergence.

Considerable genetic variation was found in the proportion of emerged plants in S1 and S2, and in the time to emergence in S1, S2 and S3. Shoot dry weight showed genetic variation in all sowings, although the coefficient of variation decreased with later sowing dates (Table 5). To trace common factors in the low-temperature response, correlations between various characteristics were considered (Table 7). The percentage of emerged plants in S1 correlates with that of S2, but there are no strong correlations with the other characteristics. Therefore reduction of emergence with early sowing has to be considered a separate phenomenon.

Time to emergence in S3 is correlated with time to emergence for the other sowing dates, which means that a common factor is involved that is largely independent of temperature. Time to emergence in S3 is correlated with intermediate plant size estimated at the 3rd-leaf stage but not with shoot dry weight at the 6th-leaf stage. This suggests that the advantage of early emergence disappeared as the seedling developed. SH1 was the genotype emerging latest in S3 and ranked second in shoot dry weight at harvest (Tables 4 and 5). Shoot growth rate after emergence seems to be more important, therefore, than rate of emergence. Path-coefficient analysis (Chapter 8) will show, however, that initial shoot growth is more important for shoot dry weight in the 6th-leaf stage than the correlations in Table 7 would suggest.

Shoot dry weight for early sowing dates is considered an important criterion for low-temperature adaptation since it is the end result of several processes. Because of the higher plant numbers in S3, its data are more reliable than the data of S1 or S2. Table 7 indicates high correlations between shoot dry weight in S3 and shoot dry weight in S2, S4 and S5. There was, however, no correlation with shoot dry weight in S6. Genetic variation in S6 was not influenced by temperature stress. There is a high correlation between the breeder's estimate for cold tolerance and shoot dry weight in S3 (r = 0.877**) which was higher or much higher than for the other sowing dates

Table 7. Correlation coefficients between characteristics of 10 single-cross hybrids.
* = $P \leqslant 0.05$; ** = $P \leqslant 0.01$.

	Number of emerged plants S1	Time to emergence S3	Shoot dry weight S3	Shoot dry weight ratio S3/S6
Kernel weight	− 0.380	0.552*	0.158	0.186
Number of emerged plants S1		− 0.204	0.249	− 0.122
Number of emerged plants S2	0.856**	− 0.191	0.132	− 0.140
Time to emergence S1	− 0.517	0.727**	− 0.122	0.371
Time to emergence S2	− 0.590*	0.690*	− 0.139	0.120
Time to emergence S3	− 0.204		0.277	0.497
Time to emergence S4	− 0.211	0.891**	0.229	0.553*
Time to emergence S5	− 0.365	0.926**	0.318	0.562*
Time to emergence S6	− 0.280	0.690*	0.352	0.672*
Emergence time ratio S3/S6	0.217	− 0.082	− 0.241	− 0.486
Resistance to chlorosis S3	0.031	0.415	0.626*	0.814**
Plant size S3 on 18 May	0.165	− 0.638*	− 0.340	− 0.724**
Shoot dry weight S1	0.294	− 0.214	0.675*	0.130
Shoot dry weight S2	0.356	− 0.017	0.918**	0.535
Shoot dry weight S3	0.249	0.277		0.737**
Shoot dry weight S4	0.270	0.421	0.920**	0.590*
Shoot dry weight S5	− 0.003	0.403	0.832**	0.508
Shoot dry weight S6	0.473	− 0.323	0.220	− 0.486
Shoot dry weight ratio S3/S6	− 0.122	0.497	0.737**	
Breeder's cold tolerance	0.016	0.578*	0.877**	0.838**

(data not presented). This is a second reason to consider the S3 data to be representative for the effects of a cool season.

Genetic variation in shoot dry weight of S6 is not caused by low-temperature stress and is, therefore, attributed to genetic differences in growth potential, or vigour, independent of temperature. These differences in seedling vigour also affected shoot dry matter accumulation in S3. Therefore, shoot dry-weight ratios S3/S6 were calculated, which may be a better indicator of the effects of low temperature than the absolute values of shoot dry weight in S3. The shoot dry-weight ratio S3/S6 ranged from 1.34 in SH1 to 0.74 in SH9 (Table 5). Again a significant difference ($P \leqslant 0.01$) was found between the flint and the dent group. High correlations were found between shoot dry weight ratio S3/S6 and resistance to chlorosis and the breeder's estimate of cold tolerance; a negative correlation was found with the plant-size estimate of S3 in the 3rd-leaf stage.

Note that correlations do not imply causal relationships. Certain independent features may coincide in genotypes as a result of selection. Selection for resistance to chlorosis and seedling vigour under low-temperature conditions may result in

genotypes that combine those characteristics. Moreover, the experiment concerns a very restricted number of genotypes, consisting of two groups viz. European flints and Corn Belt dents. Another restriction is that the results are from one experiment. Temperature conditions during this experiment were suitable, however, and the high correlations with the breeder's estimate of cold tolerance suggests the results to be representative of effects of low temperature in other years.

Pot experiment. The data of the pot experiment (Table 8) were compared with the data of the field experiment.

Average time from sowing to emergence in the pot experiment was short, 7.8 days, which is similar to the field trial S6. Genetic variation was relatively small. Time to emergence in the pot experiment correlated with that in S6 ($r = 0.780^{**}$).

Shoot dry weight in the pot experiment was compared with that in the field trial S3 since the growth period after emergence had the same weather conditions. Genetic variation was as large as it was in S3. Shoot dry weight in the pot experiment correlated with that in S3 ($r = 0.825^{**}$), but the average was 1.8 times higher. The higher shoot dry weight may be due to the slightly higher temperatures in the pot soil after emergence, although the growth period between emergence and harvest was three days shorter. Another possible cause of the higher shoot dry weight in the pot experi-

Table 8. Time to emergence (days from sowing) and shoot dry weight of 10 single-cross hybrids in a pot experiment. Data are $\bar{x} \pm s_{\bar{x}}$ of 20 plants. Growth conditions were 1 – 5 May in greenhouse, 5 May – 9 June in field. Shoot dry weight of S3 and the shoot dry weight ratio S3/pot experiment are also presented.

Genotype	Time to emergence (days)	Shoot dry weight (mg/plant)		Shoot dry weight ratio S3/pot experiment
		pot experiment	S3	
SH1	9.1 ± 0.22	956 ± 33	468	0.49
SH2	7.2 ± 0.10	961 ± 32	517	0.54
SH3	8.1 ± 0.15	665 ± 22	391	0.59
SH4	7.8 ± 0.14	592 ± 19	383	0.65
SH5	7.6 ± 0.18	586 ± 27	387	0.66
SH6	8.2 ± 0.19	643 ± 26	390	0.61
SH7	7.4 ± 0.17	568 ± 19	312	0.55
SH8	7.3 ± 0.15	620 ± 25	372	0.60
SH9	7.3 ± 0.11	532 ± 25	268	0.50
SH10	7.7 ± 0.18	726 ± 30	328	0.45
Average	7.8	685	382	0.56
Coefficient of variation	7.4	22.5	28.8	12.4

ment may be in the higher temperature and the shorter period between sowing and emergence. In that case the low shoot dry weight in S3 may be attributed to adverse after-effects of low temperature before emergence, comparable with such effects of the earlier sowings S1 and S2. The shoot dry weight ratio ½(S1 + S2)/S3 is not positively correlated, however, with the shoot dry weight ratio S3/pot experiment ($r = 0.238$). It seems likely, therfore, that other factors are involved, for instance a better soil fertility and soil structure in the pot experiment than in the field.

The first objective of the field experiment was to describe the effects of low temperature under natural conditions. We conclude that several effects can be distinguished:
– low temperature before emergence results in mortality of seeds and seedlings, malformations and delayed emergence, and it reduces seedling vigour after emergence
– low temperature after emergence causes chlorosis and reduces dry matter accumulation
Genetic variation was found for most of these effects. Chapters 3 – 6 describe experiments which give more detailed information on the above phenomena. It will be attempted to elucidate physiological backgrounds and to evaluate the significance of plant responses for maize growing and maize breeding for low temperature adaptation. Most experiments used the single-cross hybrids of the field experiment described in this chapter; in some other experiments other materials were used.

3 The effects of temperature on germination and seedling growth before emergence

3.1 Introduction

To discuss the effects of temperature on initial seedling development, a distinction will be made between imbibition, germination and early seedling growth. Germination is considered the activation of the embryo, which begins with the first metabolic activity during imbibition and ends with the emergence of the radicle from the seed (Heydecker, 1973). Early seedling growth refers to the development from radicle emergence to emergence of the coleoptile from the soil. Seedling growth after emergence will be discussed in Chapter 5.

The effect of temperature on imbibition of maize seeds has been described by Blacklow (1972a). Parameters of the imbibition curve increased with temperature. However, even at low temperature the water content of seeds increased condiderably within the first hours. It seems unlikely, therefore, that temperature restricts germination by its effect on imbibition.

The effect of temperature on germination can be expressed as its effect on time to radicle emergence or on germination rate (i.e. the reciprocal of time to radicle emergence). Blacklow (1972b) determined times of radicle emergence by linear extrapolation of time curves of radicle length to zero for several temperatures. Radicle emergence times ranged from about 6 days at 10 °C to 17 h at 32 – 34 °C. Similar data were obtained by Clegg & Eastin (1978) of time to 50 % radicle emergence. Minimum germination temperatures are in the range of 4 – 8 °C (Segeťa, 1964).

The effect of temperature on early seedling growth has been the subject of many studies. Parameters of growth are elongation rate of the primary root or the shoot at a certain stage of seedling development. In some studies, shoot length or seedling weight is determined after a fixed time of temperature exposure.

The optimum temperature for root and shoot elongation of maize seedlings is 30 – 34 °C (Sachs, 1860; Lehenbauer, 1914; Erickson, 1959; Blacklow, 1972b). Erickson (1959) measured the elongation rate of primary roots at a length of 25 mm in seedlings reared at 25 °C. He obtained a sigmoid-shaped temperature curve in which the elongation rate increased from 0.03 mm h^{-1} at 6 °C to 2.8 mm h^{-1} at 30 °C. Blacklow (1972b) studied shoot and root elongation in seedlings that were continuously exposed to a range of temperature. He found temperature curves that were close to linear between 9.5 and 30 °C. The extrapolated minimum temperature for both processes was 9 °C.

Many reports describe differential response of genotypes to low temperatures.

Segeťa (1964) investigated the effects of low temperature on germination of six maize varieties. After 28 days, percentages of seeds with coleoptiles $\geqslant$ 4 mm was 0 – 2.3 % at 4 °C, 22 – 96 % at 6 °C and 88 – 98 % at 8 °C. Roder (1962) found that time to 50 % germination at 8 – 10 °C ranged from 22 to 32 days in a group of 20 varieties. Genetic variation in percentage of germination, percentage of emergence and time to emergence at low temperature were reported by Eagles & Hadacre (1979b) and Eagles & Brooking (1981). Data of all these sources clearly show genetic variation in low-temperature response during germination and early seedling growth. However, even with the best materials the rate of the growth processes is extremely slow at temperatures around 10 °C.

The reports mentioned so far in this chapter concern physiological effects of low temperature. Deleterious effects of micro-organisms at low temperature will be discussed in Section 4.6.

This chapter records three aspects of the experimental work. First, experiments to obtain a temperature-response curve for germination and shoot elongation are described. Second, the effects of temperature and temperature fluctuations on shoot morphology are reported. Third, genetic variation in germination and emergence at low temperature is analysed for two groups of genotypes.

3.2 The effects of temperature on the rate of germination and shoot elongation in cv. Fronica

Data in the literature on the effects of temperature on germination and initial shoot elongation usually concern temperatures above 10 or 12 °C. In Lehenbauer's (1914) extensive study on shoot elongation, 12 °C was the lowest temperature used in experiments. The experiments described in this section repeat Lehenbauer's classical study, but with some modifications. First, the lowest temperature in our study was 6 °C. Second, Lehenbauer reared the seedlings at 27 – 29 °C, until the shoots had reached a length of 10 – 12 mm, before exposure to different temperatures. His data on shoot elongation at temperatures from 12 to 15 °C show a decrease of the elongation rate with time of exposure, probably due to a positive after-effect of the high rearing temperatures. In our study, seedlings were exposed to the temperatures to be studied at sowing. Third, Lehenbauer exposed his plants to weak day-light, which might have inhibited shoot elongation (Vanderhoef et al., 1979). We kept the plant material in complete darkness. Fourth, preliminary experiments showed that shoot elongation rate increased with seedling development. Therefore, we made separate estimates of the elongation rate of the first 2 cm (0 – 2 cm) and the next 2 cm (2 – 4 cm) of the shoot. Fifth, we also investigated the effects of temperature on germination.

3.2.1 Material and methods

Seeds of cv. Fronica were imbibed between moistened tissue paper at 6 °C for 2 days; a temperature of 6 °C is too low for root and shoot extension but high enough to prevent chilling injury. The imbibed seeds contained 29 g water per 100 g fresh weight of seeds. The imbibed seeds were dressed with 50 % w/w thiram and planted in pots filled with potting compost.

Four groups of pots were used:
Group A to estimate time to radicle emergence; radicles were considered emerged when their length was $\geqslant$ 2 mm.
Group B to estimate time to shoot initiation; shoots were considered initiated when the coleoptile tips were above the upper side of the kernels.
Group C to estimate time to shoot emergence of kernels planted at a depth of 2 cm.
Group D to estimate time to shoot emergence of kernels planted at a depth of 4 cm.

Each group comprised 100 seeds (10 pots of 10 seeds). The technique is illustrated in Figure 2. In all groups the seeds were placed with the embryo in a vertical position to assure regular root and shoot growth. Pots of Group A were placed upside-down on a moist cotton sheet in Petri dishes. Pots of Group B were covered with five layers of wet filter-paper to assure a sufficient water supply. Depths of 2 and 4 cm (Groups C en D) were attained by filling the pots with soil to the required sowing depth. Soil and seeds were pressed with special stamps so that the upperside of the seeds was exactly 2 or 4 cm below the rim of the pots. The pots were then completely filled with soil. The pots were placed in covered trays to reduce evaporation.

The experiment comprised 12 temperature treatments from 6 to 40 °C. Observations were made at intervals of 7 days at 6 °C through to 2 – 3 hours at 24 – 40 °C. Each time the number of emerged roots or shoots was counted. All data are based on time to 50 % emergence, which was calculated by interpolation. Shoot-elongation rates in the growth phase 0 – 2 cm and 2 – 4 cm were based on time intervals of 50 % emergence in the Groups B, C and D.

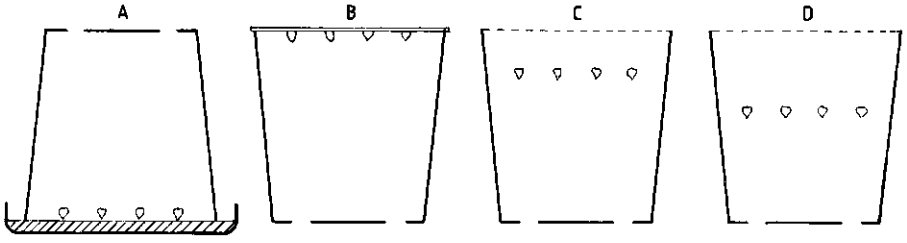

Figure 2. Diagram of the technique used. Seeds imbibed at 6 °C for 2 days were placed in pots of soil with the embryo in a vertical position; 10 seeds were planted in each pot. Group A: seeds were planted with their lower side level with the soil surface in pots that were placed upside-down on a moist cotton sheet in Petri dishes. Group B: seeds were planted with their upper side level with the soil surface and covered with filter paper. Groups C and D: seeds were planted with their upper side at 2 and 4 cm, respectively, below the soil surface.

3.2.2 Results and discussion

The effect of temperature on germination rate of roots and shoots is depicted in Figure 3. The minimum temperature for radicle emergence was about 6 °C. At 6 °C, 34 of the 100 seeds had visible radicles 44 days after sowing. With longer exposure to 6 °C this number did not increase and the emerged radicles did not elongate. At 8 °C, 50 % of the radicles had emerged after 10.6 days, and 98 % 14 days after sowing. At 8 °C, radicles were able to elongate to some extent. The root germination – temperature curve is at an optimum at 32 – 36 °C and a maximum for temperatures > 40 °C. The germination rate of shoots has a similar response to temperature. No shoots were obseved at 6 °C. At 8 °C, shoots grew to lengths of up to 10 mm. Shoot growth ceased at prolonged exposure to 8 °C, however, and no shoots emerged from the sowings at 2 and 4 cm depth. Seventy-six days after sowing seeds of Groups C and D that had been kept at 6 °C and 8 °C were exposed to room temperature. It appeared that imbibed and germinated seeds had survived those extreme treatmens. Of the material originally exposed to temperatures of 6 °C, 85 % of the seedlings emerged; of the material, originally exposed to temperatures of 8 °C, only 38 % did. In the latter case, all primary roots and most of the shoots were killed, probably by soil fungi. The reasons for the higher mortality in material exposed tot 8 °C than to 6 °C may be

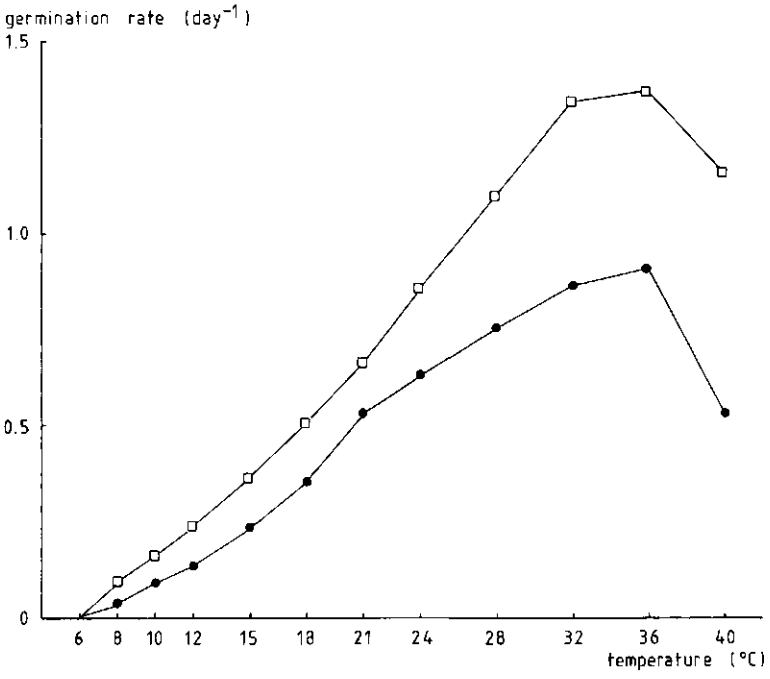

Figure 3. The effect of temperature on the germination rate of roots (□) and shoots (•) of cv. Fronica.

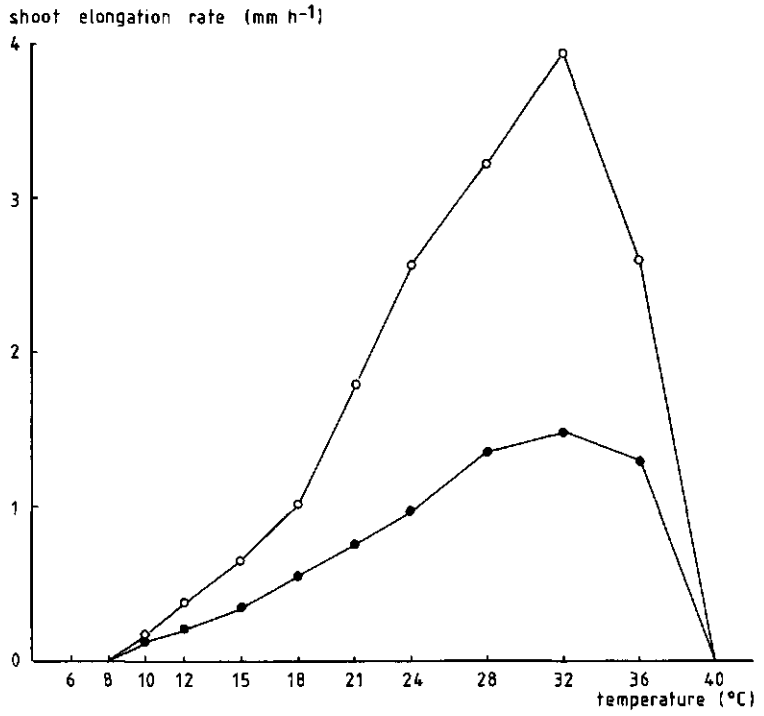

Figure 4. The effect of temperature on the elongation rate of shoots of length 0-2 cm (•) and 2-4 cm (o) of cv. Fronica.

higher activity of soil fungi at 8 °C or a more advanced stage of germination at 8 °C than 6 °C.

Shoot-elongation rates are depicted in Figure 4. Temperatures for minimum, optimum and maximum elongation were 8, 32 and 40 °C, respectively. Shoots elongate slower in the 0–2 cm phase than 2–4 cm phase; this difference increased with temperature, which is attributed to increased mesocotyl extension (Section 3.4).

Times from sowing at −4 cm tot 50 % emergence were:

Temperature (°C)	10	12	15	18	21	24	28	32	36
Days	22.9	13.9	8.0	5.1	3.8	2.8	2.2	1.9	2.1

There is a large time interval between 10 and 12 °C.

The temperature-response curve for shoot elongation from 2 to 4 cm shows two approximately linear parts, one from 8 to 18 °C and one with a steeper slope from 18 to 32 °C. A similar curve was obtained by Lehenbauer (1914) with measurements of a 9 hour temperature-exposure time. Above 32 °C, Lehenbauer (1914) also found a steep decline but the maximum temperature was 44 °C. In our study the maximum temperature for shoot elongation was 40 °C. This difference is attributed to

deleterious effects of the long exposure time in our experiment. The germination experiment (Figure 3) shows that shoots start elongation at 40 °C but growth ceases before the shoots reach a length of 2 cm.

3.3 Shoot growth and morphology at 18/6 (day/night), 12, 18 and 24 °C

Emergence, i.e. the appearance of the coleoptile tip above soil level, is the result of growth of two distinct parts of the shoot, the mesocotyl and the coleoptile. Buckle & Grant (1973) reported that low night temperatures inhibited elongation of the mesocotyl more than that of the coleoptile. This section describes an experiment to investigate the influence of temperature and temperature fluctuation on the relative contribution of mesocotyl and coleoptile plus plumule to shoot elongation and shoot dry matter accumulation around the time of emergence.

3.3.1 Material and methods

Seeds of cv. Fronica were pretreated and sown in pots at a depth of 4 cm, as described in Subsection 3.2.1. Shoot morphology and shoot growth were investigated at constant temperatures of 12, 18 and 24 °C and for diurnal fluctuations of 18/6 °C (12 h/12 h). To estimate the rate of growth processes around emergence, two groups of 10 pots (100 seeds) were used in each temperature treatment. Group I was harvested just before emergence (Harvest I) and Group II when the shoots were 1 – 2 cm above soil level (Harvest II). The material was kept completely dark until harvest. To assess the proper time of harvesting, a third group of 10 pots was used to observe shoot development. At each harvest, length of shoots, mesocotyls and coleoptiles were measured. In some cases the first leaf had broken through the coleoptile; then the length of the plumule was used instead of the length of the coleoptile. Dry weight of mesocotyls and coleoptiles with enclosed plumules was determined for each pot of 10 seedlings.

3.3.2 Results and discussion

In most cases the coleoptile enclosed the plumule, the tip of the first foliage leaf being just below the tip of the coleoptile. Sometimes growth of the plumule lagged behind that of the coleoptile, in other cases the plumule had grown through the coleoptile. The frequency of those aberrations is given in Table 9. Relative inhibition of plumule growth is mainly found at 12 °C. Of more importance is the breaking of the coleoptile by a faster growing plumule, which was observed at 18/6 °C. This phenomenon is to some extent associated with coleoptile length, since it was more frequently found in Group II than in Group I at 18/6 °C. Breaking of the coleoptile cannot be attributed to low temperatures only, as it was rarely observed at 12 °C. The phenomenon is probably caused by temperature fluctuations. Similar results were observed by Buckle & Grant (1973) at diurnal fluctuations of 27 – 32 °C (day) to 10 – 15 °C (night). When the plumules break through the coleoptile below soil level,

Table 9. Morphological aberrations of coleoptiles and plumules in seedlings of cv. Fronica germinated under four temperature regimes. Times from sowing to harvest (I, II) are presented in Table 10. Each value concerns 100 seedlings.

Temperature (°C)	Harvest	Length of coleoptile (mm/plant) $\bar{x} \pm s_{\bar{x}}$	Proportion of plumules more than 3 mm shorter than coleoptile (%)	Proportion of plumules longer than coleoptile (%)
18/6	I	21.9 ± 0.33	3	11
18/6	II	28.5 ± 0.81	6	44
12	I	16.1 ± 0.29	6	1
12	II	27.0 ± 0.40	20	1
18	I	12.5 ± 0.29	3	0
18	II	18.6 ± 0.44	2	0
24	I	11.4 ± 0.32	1	0
24	II	15.0 ± 0.46	0	0

seedlings form distorted shoots that will not emerge.

Table 10 gives data of shoot length and dry weight and mesocotyl/shoot ratios of length and dry weight. Shoot dry weight divided by shoot length decreases from 18/6 °C to 24 °C, which is attributed to a decrease in shoot diameter, mainly of the mesocotyl. For a decrease of temperature from 24 to 12 °C the proportion of mesocotyl in the shoot length decreases from about 70 % to 54 %. For a regime of 18/6 °C the proportion of mesocotyl is as low as 40 %. The dry-weight data illustrate a similar trend although the differences are less pronounced. Elongation rate and the relative growth rate between Harvest I and Harvest II are presented in Figure 5. At 12 °C the contributions of coleoptile and mesocotyl to shoot elongation are similar, whereas at 18 and 24 °C the mesocotyls elongate much faster than the coleoptiles. Relative growth rates responded to temperature similarly. The conclusion is that rapid emergence at high temperature is mainly caused by rapid elongation of the mesocotyl. However, low temperatures – and temperature fluctuations especially – cause a beneficial dry matter distribution. The high proportion of the plumule dry matter may lead to more vigourous seedlings, because the plumule is the starting material for the shoot, whereas the mesocotyls die after some time. Another beneficial effect of short mesocotyls is a good standability of the seedlings, when crown roots develop on the relatively deeply situated coleoptilar node (cf. Section 3.5).

Table 10. Shoot length, shoot dry weight, mesocotyl/shoot ratio and shoot dry weight/shoot length ratio of seedlings of cv. Fronica under four temperature regimes. The data comprise means and standard deviations of the mean ($\bar{x} \pm s_{\bar{x}}$) from average data of 10 pots containing 10 seeds.

Temperature (°C)	Time from sowing to harvest	Shoot length (mm/plant)	Length ratio mesocotyl/shoot	Shoot dry weight (mg/plant)	Dry weight ratio mesocotyl/shoot	Shoot dry weight/ shoot length (mg mm^{-1})
18/6	I 13 d, 2.5 h	37.8 ± 0.71	0.419 ± 0.005	13.4 ± 0.25	0.348 ± 0.006	0.356 ± 0.006
18/6	II 14 d, 20.5 h	47.1 ± 1.45	0.394 ± 0.005	16.7 ± 0.53	0.329 ± 0.005	0.354 ± 0.004
12	I 12 d, 21 h	35.4 ± 0.86	0.544 ± 0.006	10.5 ± 0.24	0.460 ± 0.004	0.298 ± 0.002
12	II 14 d, 21 h	59.0 ± 0.16	0.540 ± 0.004	16.6 ± 0.24	0.461 ± 0.004	0.281 ± 0.004
18	I 4 d, 17 h	30.6 ± 0.96	0.588 ± 0.006	8.4 ± 0.25	0.464 ± 0.008	0.274 ± 0.003
18	II 5 d, 6 h	51.9 ± 1.09	0.642 ± 0.003	12.9 ± 0.26	0.496 ± 0.005	0.249 ± 0.004
24	I 2 d, 16.5 h	33.3 ± 1.36	0.656 ± 0.007	8.3 ± 0.23	0.476 ± 0.007	0.249 ± 0.004
24	II 2 d, 22.5 h	50.5 ± 1.58	0.702 ± 0.006	11.4 ± 0.38	0.512 ± 0.007	0.226 ± 0.002

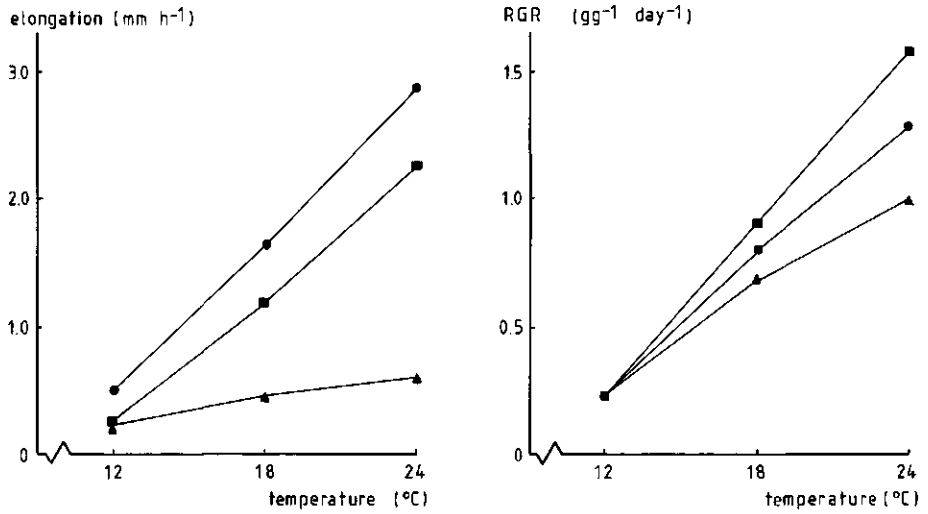

Figure 5. The effect of temperature on elongation rate and the relative growth rate (RGR) of coleoptile plus plumule (▲), mesocotyl (■) and total shoot including the mesocotyl (●) in seedlings of cv. Fronica.

3.4 Germination and emergence of some landraces and CIMMYT gene-pool populations at 12 °C

Germination and emergence at low temperatures are considered selection criteria for adaptation of maize to a cool climate. Genetic variation has been reported by several authors (Section 3.1).

The aims of the following experiment were:

- to investigate time to germination and time to emergence at 12 °C in a group of exotic materials and European landraces
- to investigate relationships between germination and emergence
- to study shoot morphology of the materials in relation with emergence.

A temperature of 12 °C was chosen because it is just above the minimum temperature for germination, even for non-adapted materials.

3.4.1 Materials and methods

Twelve accessions of maize were investigated. The materials consisted of seven landraces of tropical and temperate origin, two CIMMYT gene-pool populations for tropical highland, two CIMMYT gene-pool populations for tropical lowland, and the Dutch hybrid variety Fronica (Table 11). Those materials were also used in the experiments on seedling growth after emergence reported in Chapter 7.

Germination and emergence experiments were done at 12 °C. The experiments were

started with dry seeds. For germination experiments, five Petri dishes with 10 seeds of each accession were used; the seeds were kept in the dark between moist tissue papers. Emergence and shoot morphology were investigated on seedlings grown in pots containing potting compost. The seeds were dressed with 50 % (w/w) thiram and sown in a vertical position at a dept of 4 cm. Five pots with 10 seeds were used per accession. To assure normal shoot development this experiment was carried out in continuous fluorescent light of an intensity (400 – 700 nm) of about 20 W m^{-2}. Time to 50 % germination, i.e. radicle emergence, and time to 50 % emergence were estimated by observations two times a day. In the experiment on emergence and shoot morphology, shoots were harvested 20 days after sowing. Lengths of shoot, coleoptile plus plumule, and mesocotyl were measured for individual seedlings. Dry weight of coleoptile plus plumule and mesocotyl was estimated for each pot, containing 10 seedlings. Non-germinated seeds were omitted in the calculation of the means. Correlation coefficients between time to germination, time to emergence and shoot characteristics were calculated.

3.4.2 Results and discussion

The main results are given in Table 11. The first radicles became visible in materials No 2 and No 12, 4 days after sowing. The germination experiment was terminated 10 days after sowing. At that time germination percentages ranged from 86 to 100 %. The average time to 50 % germination was 6.5 days; the difference between the material germinating earliest (No 2) and latest (No 10) was 3 days.

The average time to emergence was 17.7 days. The earliest material (No 12) emerged about 5 days before the latest (No 8). The first 4 cm of the shoot had grown below soil level, in the dark. Some time after emergence, exposure to light stopped extension of the coleoptiles and the plumules broke through. Only in materials No 7 and No 8 did some plumules (12 % in both) grow through the coleoptile below soil level. Coleoptile length (not presented in Table 11) showed little variation; average length was 26.3 mm, ranging from 21.6 mm in No 3 to 32.3 mm in No 12 material. Shoot length ranged from 44.8 mm in No 8 material to 81.7 mm in No 12. Mesocotyl length was about 30 to 40 mm for most of the materials; extremely short mesocotyls were found in the tropical lowland materials, No 7 and No 8, and also in material No 2. At the time of harvesting, on average half the amount of shoot dry matter was in the plumule and coleoptile. Dry weight of the plumule is significant because it is the starting biomass of the shoot. Dry weight of the plumule plus coleoptile ranged from 7.2 mg in material No 2 to 18.9 mg in material No 12; the tropical lowland materials, No 7 and No 8 had, relatively large plumules.

Correlation coefficients are given in Table 12. Time to germination correlated with none of the other characteristics except plumule length. Time to emergence is strongly correlated with shoot length and also with mesocotyl length, but not with length of coleoptile or plumule. Shoot length is correlated with mesocotyl length and dry weight, but not with plumule length. Shoot dry weight is correlated with plumule dry weight, but not significantly with dry weight of the mesocotyl.

Table 11. Time to germination and emergence, and shoot characteristics of 12 maize accessions exposed to a temperature of 12 °C. Length and dry weight of shoots and shoot parts were determined 20 days after sowing. Values are means of 5 replicates of 10 seeds or plants each.

Materials (and their origin)	Time (days) to		Length (mm/plant)		Dry weight (mg/plant)	
	50 % germination	50 % emergence	shoot	mesocotyl	shoot	plumule + coleoptile
1 Conico, Zacatecas 34 (Mexico)	6.3	16.7	62.9	35.8	18.3	8.3
2 Chapalote-Reventador, Sinaloa 2 (Mexico)	4.8	18.3	54.2	17.3	14.8	9.6
3 Chalqueño, Mexico Grupo 17 (Mexico)	6.7	15.9	62.7	39.1	18.6	7.1
4 Tuxpeño, Queretaro Grupo 17 (Mexico)	5.8	15.7	70.7	36.5	21.6	10.8
5 CIMMYT gene pool 4, HEYF	6.5	15.6	70.3	36.7	21.5	11.4
6 CIMMYT gene pool 5, HEYD	6.5	16.3	66.9	36.8	21.7	10.8
7 CIMMYT gene pool 21, LIYF	6.4	19.5	47.8	13.6	19.9	14.2
8 CIMMYT gene pool 22, LIYD	6.7	19.7	44.8	11.6	17.2	12.4
9 Branco de Pias (Portugal)	7.7	17.7	59.5	35.2	22.9	10.4
10 Rheintaler (Switzerland)	7.8	17.3	59.3	31.8	21.0	10.0
11 Mestnaja scorospelaja (USSR)	6.9	17.6	58.0	30.8	16.8	8.0
12 cv. Fronica	5.5	14.4	81.7	29.7	28.8	18.9
Average	6.5	17.7	61.6	29.6	20.3	11.0

Mesocotyl length and dry weight are strongly correlated, like plumule length and dry weight. Therefore length measurements can be replaced by an estimate of dry weight (Section 3.5).

It can be concluded that at 12 °C, genetic variation of time to emergence and time to germination are independent. Time to emergence is closely related to mesocotyl extension, but it has no relationship with dry weight of the plumule.

Note that the above relationships concern the material chosen for this experiment. Figure 6 shows the relationship between time to germination and time to emergence. Three of the accessions, materials No 2, 7 and 8, differ from the other materials: they are characterized by short mesocotyls (Table 11). Short mesocotyls are probably typical for tropical lowland maize.

3.5.2 Results and discussion

The results of this experiment are given in Table 13. Time to emergence ranged from 14.0 days in SH2 to 18.3 days in SH4. Shoot dry weight ranged from 14.8 mg in SH3 to 29.8 mg in SH6, and dry weight of mesocotyl from 6.6 mg in SH3 to 12.6 mg in SH6. Those ranges were only slightly smaller than in the previous experiment (see Table 11).

Correlation coefficients are given in Table 14. Shoot dry weight is strongly correlated with dry weight of plumules and mesocotyls; in the previous experiment

Table 13. Time to emergence and dry weight of plumule (plus coleoptile), mesocotyl and total shoot of 10 single-cross hybrids and two cultivars grown at 12 °C for 19 days. Data are the means of four replicates of 10 plants.

Genotype	Time to emergence (days)	Dry weight (mg/plant)		
		plumule	mesocotyl	shoot
SH1	17.5	13.0	9.6	22.6
SH2	14.0	14.5	9.2	23.7
SH3	17.8	8.2	6.6	14.8
SH4	18.3	8.6	6.8	15.4
SH5	17.2	10.0	8.7	18.7
SH6	17.0	17.0	12.8	29.8
SH7	15.7	14.2	12.0	26.2
SH8	15.5	11.4	10.5	21.9
SH9	18.0	11.7	9.6	21.3
SH10	17.2	12.1	10.0	22.1
Eta Ipho	15.1	13.0	8.4	21.4
Fronica	15.6	13.9	8.7	22.6
Average	16.6	12.3	9.4	21.7

Table 14. Correlation coefficients between time to emergence and shoot characteristics at 12 °C and date of field emergence (Chapter 2, sowing date S3) of 10 single-cross hybrids and two varieties. Data from Tables 13 and 4. * = $P \leqslant 0.05$ ** = $P \leqslant 0.01$

	1	2	3	4	5
1 Time to emergence	1.000				
2 Dry weight of plumule	−0.533	1.000			
3 Dry weight of mesocotyl	−0.252	0.790**	1.000		
4 Dry weight of shoot	−0.439	0.963**	0.926**	1.000	
5 Emergence date in field, 1975[a]	0.576*	−0.323	−0.377	−0.356	1.000

[a] Characteristics of 10 single-cross hybrids only

(Table 12) the correlation coefficients between those characters were much lower. Time to emergence did not significantly correlate with the shoot characteristics, in constrast to the previous experiment, in which correlations with dry weight of shoot and mesocotyl were found. For the group of 10 single-cross hybrids, a significant but low correlation was found between time to emergence at 12 °C in the laboratory and time to emergence of early sowing (S3) in the field.

It can be concluded that with early seedling growth at 12 °C, no close relationships were found between time to emergence and shoot characteristics such as mesocotyl growth.

3.6 Discussion and conclusions

Temperature-response curves for germination and initial shoot elongation (Figures 3 and 4) were similar to those reported in the literature. Germination and shoot elongation were very slow at 8 – 10 °C. Shoot elongation rate at a given temperature was not constant but increased with shoot development.

Temperature also affected shoot morphology (Section 3.3.). Low temperatures resulted in seedlings with relatively short mesocotyls, since mesocotyl extension is more impeded by low temperatures than plumule growth is; large diurnal temperature fluctuations had a similar or even stronger effect (see also Buckle & Grant, 1974). This implies that data from laboratory experiments at constant temperatures cannot be extrapolated to field conditions, where there are diurnal temperature fluctuations. Another morphogenetic effect of temperature fluctuations (see Table 9, 18/6 °C) was plumule growth through the coleoptile, which may be attributed to different extension rates of the two organs. In the field this may decrease the number of emerging plants.

Genotypes adapted to tropical lowlands had extremely short mesocotyls under low temperature conditions (Table 11; see also Chapter 7). It may be assumed that such materials have a normal mesocotyl length under tropical conditions, with high temperatures and little diurnal fluctuation, whereas mesocotyls of varieties from temperate regions become too long under such conditions (Table 10, 24 °C).

In maize breeding much attention is paid to the effects of low temperature on the rate of germination and emergence. Laboratory tests at constant low temperatures are often used to characterize genotypes. The value of the results is questionable, however, for several reasons. First, diurnal temperature fluctuations should be used instead of constant temperatures. Second, there is insufficient evidence to support the presumption that rapid germination and emergence are beneficial for seedling growth. Selection for rapid emergence may lead to genotypes with relatively long mesocotyls and small plumules. Such seedlings have a small shoot biomass and a poor standability.

Table 15. The effect of chilling on germination, early seedling growth and root necrosis in 10 single-cross hybrids, planted in moist perlite. Treatments: A. 6 d at 20 °C; B. 6 d at 2 °C, then 6 d at 20 °C; C. 3 d at 20 °C, then 6 d at 2 °C, then 3 d at 20 °C. Data are means of 40 seeds of each genotype except SH1; its data are means of 20 seeds. Data of Bs are means of plants of Treatment B with normal shoots (length > 10 mm). Root necrosis was assessed on a 1 – 9 scale: 1 is most necrotic and 9 is least necrotic.

Geno-type	Proportion of plants with shoots > 10 mm (%)			Shoot length mm/plant				ratio C/A	Root length mm/plant				ratio C/A	Resistance to root necrosis in C $\bar{x} \pm s_{\bar{x}}$
	A	B	C	A	B	Bs	C		A	B	Bs	C		
SH1	100	100	100	41	65	65	21	0.51	104	142	142	38	0.37	8.8 ± 0.25
SH2	100	100	100	63	94	94	27	0.43	130	130	130	54	0.42	9.0 ± 0.00
SH3	98	62	100	55	48	77	23	0.42	108	70	111	34	0.31	5.9 ± 0.29
SH4	100	90	100	43	61	66	21	0.49	92	85	95	28	0.30	5.8 ± 0.25
SH5	98	70	100	59	45	65	21	0.36	94	64	92	23	0.24	5.6 ± 0.18
SH6	100	100	100	57	90	90	27	0.47	114	131	131	27	0.24	4.2 ± 0.16
SH7	100	95	100	53	75	78	28	0.53	78	77	80	27	0.35	6.0 ± 0.33
SH8	100	98	100	66	90	92	27	0.41	106	115	115	25	0.24	3.9 ± 0.23
SH9	100	100	100	64	84	84	28	0.44	124	132	132	23	0.19	2.1 ± 0.29
SH10	100	100	100	62	90	90	25	0.40	136	134	134	23	0.17	1.1 ± 0.12
Mean	99.6	91.5	100	56.3	74.2	80.1	24.8	0.45	108.6	108.0	116.2	30.2	0.28	5.24
Coefficient of variation	0.8	15.2	0.0	15.2	24.8	14.5	12.1	11.9	16.6	28.2	18.2	32.2	28.8	48.5

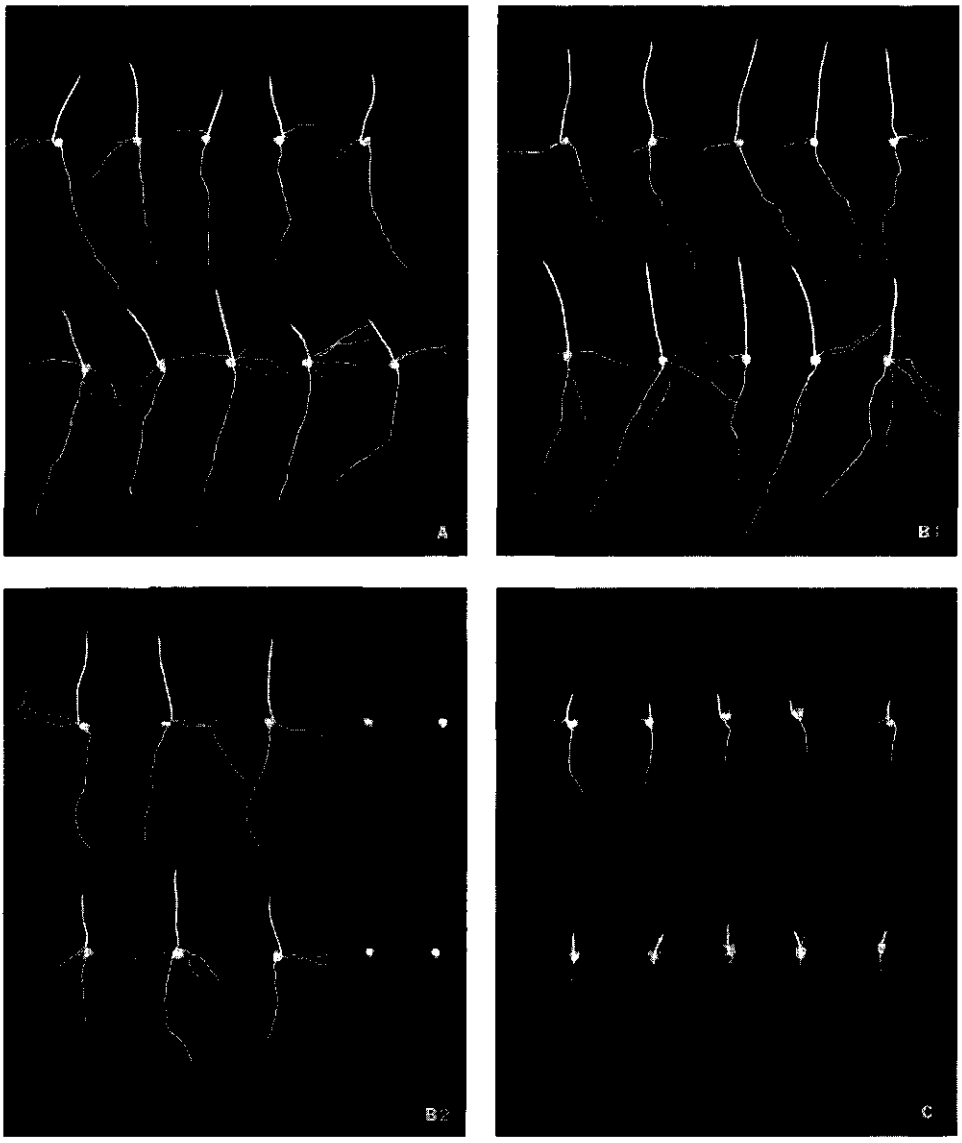

Figure 7. Seedlings at the end of the chilling treatments. Treatment A: control, 6 days at 20 °C. Treatment B: 6 days at 2 °C and 6 days at 20 °C. Treatment C: 3 days at 20 °C, 6 days at 2 °C and 3 days at 20 °C. In Treatments A, B(1) and C genotypes used: SH2 in top row; SH10 in bottom row. In Treatment B(2) genotypes used: SH3 in top row; SH5 in bottom row.

Table 16. Correlation coefficients between plant characteristics of 10 single-cross hybrids of the chilling experiment (1 – 4) and between chilling resistance parameters and plant characteristics of the field experiment of Chapter 2 (5 – 15). * = $P \leqslant 0.05$ ** = $P \leqslant 0.01$

1	Root length ratio C/A	–	Shoot length ratio C/A	0.492
2	Resistance to root necrosis C	–	Percentage germinated seeds B	–0.133
3	Resistance to root necrosis C	–	Shoot length ratio C/A	0.362
4	Resistance to root necrosis C	–	Root length ratio C/A	0.946**
5	Percentage germinated seeds B	–	Number emerged plants S1	0.338
6	Percentage germinated seeds B	–	Number emerged + germinated plants S1	0.655*
7	Resistance to root necrosis C	–	Number emerged plants S1	–0.059
8	Resistance to root necrosis C	–	Number emerged + germinated plants S1	–0.064
9	Resistance to root necrosis C	–	Time to emergence S3	0.419
10	Resistance to root necrosis C	–	Dry weight ratio ½(S1 + S2)/S3	–0.100
11	Resistance to root necrosis C	–	Resistance to chlorosis S3	0.583*
12	Resistance to root necrosis C	–	Plant size S3 on 18 May	–0.425
13	Resistance to root necrosis C	–	Shoot dry weight S3	0.823**
14	Resistance to root necrosis C	–	Shoot dry weight S3/S6	0.781**
15	Resistance to root necrosis C	–	Breeder's cold tolerance	0.928**

root-length ratio because necrosis affected root elongation. No correlation was found between imbibitional chilling injury (percentage of germinated seeds in Treatment B) and root necrosis.

The two main chilling-resistance parameters are the percentage of germinated seeds in Treatment B and resistance to root necrosis. Correlation coefficients between those parameters and plant responses in the field experiment of Chapter 2 are also presented in Table 16. The percentage of germinated seeds after imbibitional chilling did not correlate with the number of emerged plants of the first sowing date, although a significant but rather low correlation was found with the number of emerged plus germinated plants. Imbibitional chilling injury probably affected only SH5 in the field experiment (Table 3). Either no or only low correlations were found between resistance to root necrosis and the numbers of emerged or germinated plant in S1, time to emergence in S3, the shoot dry weight ratio ½(S1 + S2)/S3 and resistance to chlorosis. However, a high correlation was found between resistance to root necrosis and shoot dry weight of S3, shoot dry weight ratio S3/S6 and also the breeder's estimate of cold tolerance. The effect of chilling treatments before emergence on seedling growth after emergence was investigated in the experiments described in Section 4.4.

The correlation coefficients between imbibitional chilling injury, root necrosis, field emergence and chlorosis suggest that these characteristics are largely independent. The relationship between chilling-induced root necrosis and seedling growth at

low temperature deserves further investigation. In Chapter 7, experiments are described that investigate this relationship in a group of exotic accessions.

4.3 The effect of cold acclimation and stage of seedling development on chilling sensitivity

In the previous experiment, germinated seeds that were chilled 3 days after exposure to 20 °C were most sensitive to chilling. Wheaton (1963) showed that tomato seedlings became less sensitive to chilling when they were hardened at 12.5 °C for 1 – 8 days. Hetherington et al. (1983) showed that leaves of maize seedlings after emergence could be chill-hardened by exposure to 16/6 °C (day/night) for 17 days. Chill-hardening might have occurred in the early sowings of the field experiment, reported in Chapter 2, so we investigated the effect of cold acclimation on chilling sensitivity. An acclimation temperature of 10 °C was chosen because this is just above the minimum temperature for germination. At 10 °C, seedling growth continued at a slow rate, so that seedling size increased with the duration of the acclimation treatment. A second experiment was carried out to investigate chilling sensitivity in seedlings of varying stages of development.

4.3.1 Materials and methods

The treatments of the experiments are given in Table 17.

The cold acclimation experiment was done with four single-cross hybrids (SH2, SH7, SH9 and SH10) that have large differences in chilling sensitivity. Seeds were first germinated at 20 °C for 3 days. The just germinated seedlings were acclimated to cold at 10 °C for 0, 2, 4 and 6 days (Treatments A, B, C and D, respectively), then chilled at 2 °C for 6 days and finally exposed to 20 °C for 3 days.

The effect of the stage of seedling development was investigated with two single-cross hybrids (SH7 and SH10). Seedlings of different stages were obtained by exposing seeds to 20 °C for 0, 1, 2, 3 and 4 days (Treatments E, F, G, H and I, respectively). These seedlings were acclimated to cold at 10 °C for 4 days, and than chilled at 2 °C for 6 days. Finally they were exposed to 20 °C for 6, 5, 4, 3 and 2 days, respectively, so that the total exposure tot 20 °C was 6 days in all treatments.

The technique used is similar to that described in Subsection 4.2.1. Initial shoot length was measured after the exposure to 2 °C and final shoot length at the end of the treatments. Root and shoot necrosis was also estimated at the end of the treatments.

4.3.2 Results and discussion

The results of the acclimation experiment are presented in Table 17, Treatments A – D. Exposure to 10 °C increased initial shoot length. Final shoot lengths were hardly affected but necrosis of roots increased with longer 10 °C pre-treatments. With 4 and 6 days of 10 °C pre-treatment even shoots were affected by chilling.

Table 17. The effect of cold acclimation and stage of seedling development on chilling sensitivity. Treatments A-D investigate the effect of acclimation at 10 °C after germination and before chilling in genotypes SH2, SH7, SH9 en SH 10. In Treatments E-I, seeds of SH7 and SH10 in various stages of germination are first exposed to 10 °C for 4 days and then to the chilling treatment. In all treatments total duration of exposure to 20 °C was 6 days. Initial and final shoot length were measured at the end the 2 °C exposure and at the end of the whole treatment, respectively. Twenty seeds of each genotype were used per treatment.

	Duration of exposure to given temperature (days)				Initial shoot length (mm)				Final shoot length (mm)				Number of necrotic roots				Number of necrotic shoots			
	20 °C	10 °C	2 °C	20 °C	SH2	SH7	SH9	SH10	SH2	SH7	SH9	SH10	SH2	SH7	SH9	SH10	SH2	SH7	SH9	SH10
A	3	–	6	3	2.4	1.5	1.6	2.4	33.2	35.0	34.0	32.3	1	4	16	17	0	0	0	0
B	3	2	6	3	6.6	5.3	6.1	7.0	35.4	33.7	32.2	30.8	3	7	18	19	0	0	0	0
C	3	4	6	3	7.3	7.0	8.0	9.4	36.2	32.2	32.8	27.7	5	11	19	19	0	5	3	13
D	3	6	6	3	10.9	10.1	12.1	12.5	37.4	34.8	35.9	31.8	5	12	19	20	0	8	3	17
E	–	4	6	6		1.0		1.0		115.6		108.2		0		0		0		0
F	1	4	6	5		1.4		1.8		85.1		72.2		0		0		0		0
G	2	4	6	4		3.4		4.2		47.6		44.3		8		20		0		1
H	3	4	6	3		7.8		10.5		38.4		36.8		17		20		11		13
I	4	4	6	2		17.3		23.8		42.0		43.3		19		20		6		18

Genotypes clearly differed in extent of root necrosis, as they did in the previous experiment (Table 15).

The effect of the stage of seedling development on chilling sensitivity can be seen from the data of Table 17, Treatments E – I. Final shoot length decreases if the germination time is increased from 0 to 3 days. The number of necrotic roots and shoots increases with the stage of development of the seedlings.

No beneficial effect of cold acclimation was demonstrated. Chilling sensitivity strongly increased with seedling development.

4.4 The effect of chilling before emergence on seedling growth after emergence in SH5 and SH10

Chilling treatments after germination depressed early seedling growth and caused root necrosis in sensitive genotypes. We then investigated the effect of chilling treatments before emergence on seedling growth after emergence for two single-cross hybrids that differed in chilling resistance.

4.4.1 Material and methods

Single-cross hybrids SH5 and SH10 were used; their resistance to root necrosis was 5.6 and 1.1, respectively. Seeds were sown in pots (diameter 16 cm) containing potting compost. The experiment was carried out in a greenhouse. Throughout the experiment average temperature was 17 °C; the average daily maximum was 21 °C and the minimum 13 °C. The growth period was interrupted by chilling treatments of 2 and 4 °C, 3 and 5 days after sowing, respectively (Table 18). Dry weight of the plants was estimated after 20 days of exposure to the greenhouse conditions. At that time the seedlings were in a 4th to 5th leaf stage.

4.4.2 Results and discussion

The results are presented in Table 18. The results should be considered preliminary as only 10 plants were used per treatment and no more than two genotypes were tested. Shoot dry weight of all chilling treatments was lower than that of the control. Seedling growth was more reduced with 2 °C than with 4 °C chilling. Chilling 5 days after sowing caused more growth reduction than 3 days after sowing. In all chilling treatments, seedling growth was more reduced in SH10 than in SH5, which concurs with their sensitivities to root necrosis (Table 15). However no correlation was found between reduction of shoot dry weight by early sowing in the field (dry weight ratio ½(S1 + S2)/S3) and resistance to root necrosis with chilling (Table 16).

It seems that artificial chilling after germination may reduce seedling growth but there is no evidence for such an after-effect under field conditions.

Table 18. The effect of chilling treatments before emergence on shoot dry weight of single-cross hybrids SH5 and SH10. Seedlings were harvested after 20 days of exposure to greenhouse conditions; 10 plants of each genotype were used per treatment.

Treatment	Shoot dry weight (mg/plant) $\bar{x} \pm s_{\bar{x}}$	
	SH5	SH10
Control, 20 days greenhouse	125 ± 2.7	126 ± 6.9
Chilling 6 d 4 °C, 3 d after sowing	119 ± 2.3	119 ± 3.5
Chilling 6 d 2 °C, 3 d after sowing	115 ± 5.0	107 ± 5.2
Chilling 6 d 4 °C, 5 d after sowing	94 ± 2.7	93 ± 6.3
Chilling 6 d 2 °C, 5 d after sowing	81 ± 2.3	72 ± 3.6

4.5 After-effects of various low temperature treaments in cv. Fronica

Reduced plant vigour at early sowing, expressed by the shoot dry weight ratio ½(S1 + S2)/S3, was not correlated with genetic variation in chilling resistence (Table 16). Another explanation might be the reduction of seed reserves by respiration at low temperatures. Therefore, we investigated the effect of different low temperature treatments on the decrease of seed weight between sowing and emergence. Effects of these treatments on shoot dry matter accumulation after emergence were also investigated, for two temperature regimes.

4.5.1 Material and methods

The experiment was done with cv. Fronica. Seeds were sown in pots (16 cm diameter) containing potting compost. The pots were exposed to four treatments:
A. Control; 6 days at 20 °C.
B. 14 days diurnal fluctuation of 16/10 °C (12h/12h), then 1 day at 20 °C.
C. 14 days at 10 °C, then 3 days at 20 °C.
D. 14 days diurnal fluctuation of 10/4 °C (12h/12h), then 4 days at 20 °C.
At the end of treatments 80 – 90 % of the plants had emerged, so that all materials were at about the same stage of development. Three groups of 40 seeds (eight pots with five seeds) were used per treatment. Initial dry weight of seeds was determined per pot of five seeds. At the end of the treatment, one group was harvested to determine dry weight of seeds and dry weight of the shoots before further treatments; the shoots were cut off at the coleoptilar node. The second group of pots was transferred to a growth cabinet at temperatures of 16/10 °C, and the third group to a growth cabinet at temperatures of 25/15 °C. In the growth cabinets, light intensity (400 – 700 nm)

was 55 W m^{-2}, day length 16 h and relative humidity 70 %. Plants were harvested after 10 days at 16/10 °C and 4 days at 25/15 °C; the plants were in their 3rd-leaf stage. Dry weight of shoots was determined per group of five plants. Relative growth rate of the shoot (RSGR) was calculated from initial and final shoot dry weight.

4.5.2 Results and discussion

The effects and after-effects of the low temperature treatments can be seen from the data given in Table 19. Decrease of seed weight was nearly equal in all treatments, so exposure to low temperature during germination did not cause extra loss of seed reserves in this experiment.

The shoot dry weight at emergence was about 10 mg after the four treatments, so that the growth after emergence started at similar stages of seedling development. Standard deviations of the mean of initial and final shoot dry weight were rather high. Relative growth rates of the shoot after emergence seemed to be little affected by the treatments.

It can be concluded that exposure of germinating seeds to low temperatures above the chilling range hardly affected seedling growth after emergence. However, the duration of the period from sowing to emergence was much shorter than in the early sowings reported in Chapter 2. The length of this period concurs with farming conditions in the Netherlands.

4.6 Survival of ten single-cross hybrids at low temperature in pathogenic soil (cold test)

In the preceding sections, perlite or sterilized potting compost were used in the low temperature experiments to avoid the effects of soil fungi. No relationship was found between the effects of chilling treatments and survival in the early sowings of Chapter 2. In the experiment described in this section, it was attempted to reproduce soil conditions in the field to investigate the effect of soil fungi at low temperature. In standard cold tests seeds are planted in pathogenic soil at a temperature of 10 °C for one or two weeks. Preliminary cold tests at 10 °C for 20 days showed 100 % survival even in genotypes with poor field emergence, whereas cold tests at alternating temperatures of 4 °C and 10 °C for four weeks reduced survival to 70 – 75 % for sensitive genotypes. In the experiment, seeds in pathogenic soil were exposed to alternating temperatures of 5 and 10 °C for five weeks; the lowest temperature was 5 °C, to avoid chilling injury.

4.6.1 Material and methods

This experiment was carried out in 1980. Seeds of the 10 single-cross hybrids were taken from the stock used in Chapter 2. The seeds had been stored under optimum conditions (2 °C, 30 % relative humidity).

Table 19. Effects and after-effects of low temperature treatments before emergence in cv. Fronica. Initial seed dry weight was 288 mg; data are $\bar{x} \pm s_{\bar{x}}$ of 8 pots of 5 plants each.

Treatment	Proportion of decrease of seed dry weight (%) during treatment	Shoot dry weight (mg/plant) at emergence	Final shoot dry (mg/plant)		RSGR ($g\ g^{-1}\ d^{-1}$)	
			10 d 16/10 °C	4 d 25/15 °C	16/10 °C	25/15 °C
6 d 20 °C (control)	18.3 ± 0.47	10.1 ± 0.68	57.4 ± 1.4	73.6 ± 2.3	0.174	0.497
14 d 16/10 °C, 1 d 20 °C	18.5 ± 0.51	10.3 ± 0.77	55.1 ± 0.9	63.6 ± 1.7	0.168	0.455
14 d 10 °C, 3 d 20 °C	17.0 ± 0.78	11.2 ± 1.27	58.3 ± 2.6	83.7 ± 3.6	0.165	0.503
14 d 10/4 °C, 4 d 20 °C	17.2 ± 0.34	10.0 ± 0.72	57.2 ± 1.9	71.2 ± 1.6	0.174	0.491

Seeds were dressed with (w/w) thiram 50 % and sown in a vertical position at a depth of 4 cm in pots (diameter 12 cm) containing pathogenic soil from a field in which maize had been grown. Ten seeds were sown per pot; four pots (40 seeds) were used per genotype, although only 20 seeds were used for SH1. After sowing, the pots were exposed to low temperatures, alternating four days at 5 °C and three days at 10 °C, for a period of five weeks. Then the pots were exposed to a constant temperature of 15 °C and continuous light of an intensity of 20 W m^{-2} (400 – 700 nm). During exposure at 15 °C emergence was recorded daily. After 14 days at 15 °C, the experiment was stopped and the survival of seedlings that had not emerged was investigated.

4.6.2 Results and discussion

The results are given in Table 20. Time from sowing to emergence was on average 43 days; in S1 and S2 of the 1975 field experiment this time was 53 and 37 days, respectively (Table 4). The average percentage of emerged plants was similar to that of S2 (Table 3), but genetic variation was much smaller in the cold test than in the first two sowings (S1 and S2) of the field experiment. Striking differences were found for SH5 en SH7: they showed a much better emergence in the cold test than in the 1975 field experiment. Correlation coefficients between cold test and average data of S1 and S2 of the field experiment for time to emergence, percentage of emerged plants and percentage of surviving plants are 0.287, – 0.104 and 0.129, respectively. The correlations

Table 20. Effect of exposure to low temperature on seeds of 10 single-cross hybrids in pathogenic soil. Treatment: 5 weeks 5 °C and 10 °C (alternating 4 d 5 °C and 3 d 10 °C), then 14 d 15 °C. Forty seeds were used for genotypes SH2-SH10, and 20 seeds for SH1.

Genotype	Time to emergence at 15 °C (days), $\bar{x} \pm s_{\bar{x}}$	Proportion of emerged plants (%)	Proportion of surviving plants (%)
SH1	11.2 ± 0.29	47.5	60.0
SH2	8.0 ± 0.32	65.0	90.0
SH3	8.2 ± 0.17	57.5	65.0
SH4	9.2 ± 0.19	85.0	90.0
SH5	8.1 ± 0.21	75.0	75.0
SH6	8.1 ± 0.16	60.0	65.0
SH7	8.2 ± 0.18	62.5	65.0
SH8	7.3 ± 0.20	82.5	82.5
SH9	10.9 ± 0.25	67.5	87.5
SH10	9.0 ± 0.22	60.0	62.5
Average	8.0	66.2	74.2

are low and not significant. We concluded that the genetic variation in survival in S1 and S2 of the field experiment of 1975 (Chapter 2) could not be reproduced by the above cold test. Similar results were obtained with other cold-test treatments.

4.7 The effects of various seed dressings

Two types of dressings were used in the various experiments on low temperature damage before emergence. In the 1975 field experiment we used a mixture of the fungicide thiram 25 % (w/w) and the insecticides methiocarb 25 % (w/w) and lindane 10 % (w/w), whereas in the chilling and cold-test trials described so far we only used thiram 50 % (w/w). Different effects of low temperature might have been associated with seed dressing. Nesić & Delević (1975) reported adverse effects of lindane on germination of maize under cold-test conditions. Therefore the effects of various seed dressings at low temperature have been tested.

4.7.1 Material and methods

Three experiments were carried out:

Experiment 1. Seeds of two varieties cv. Campo and cv. Onix 95 were treated with six dressings of thiram, methiocarb, or lindane separately or in combination, denoted by B – G in Table 21. Seeds were planted in moist perlite and exposed to two temperature regimes: 20 days at 10 °C, followed by 3 days at 20 °C, and 5 days at 20 °C. At the end of the temperature treatments, length of the shoot and the primary root were measured.

An additional trial was carried out with the single-cross hybrids SH5, SH7, SH8 and SH10. Two seed dressings, B and F (see Table 21) were tested. The seeds were planted in pathogenic soil at a depth of 1 cm and exposed to 10 °C for 19 days and then 20 °C for 2 days. Twenty seeds of each genotype were used per seed treatment. In this trial only shoot length was measured.

Experiment 2. This experiment was a part of the cold-test trial of Section 4.6. Four single-cross hybrids, SH4, SH5, SH7 and SH9, were tested for the effects of seed dressings B and G (Table 21), the latter being the seed dressing in the 1975 field experiment.

Experiment 3. The effects of seed dressings B and F (Table 21) were tested under normal field conditions. Seeds of the single-cross hybrids SH2, SH9 and SH10 were planted at a depth of 5 cm in pots with soil from the field of the 1975 experiment. Sowing date was 25 April 1979. The pots were placed in the field. Date of emergence was recorded daily. Shoot length above soil level was measured on 12 May. From 25 April to 12 May average daily maximum and minimum temperature were 18 and 6 °C, respectively.

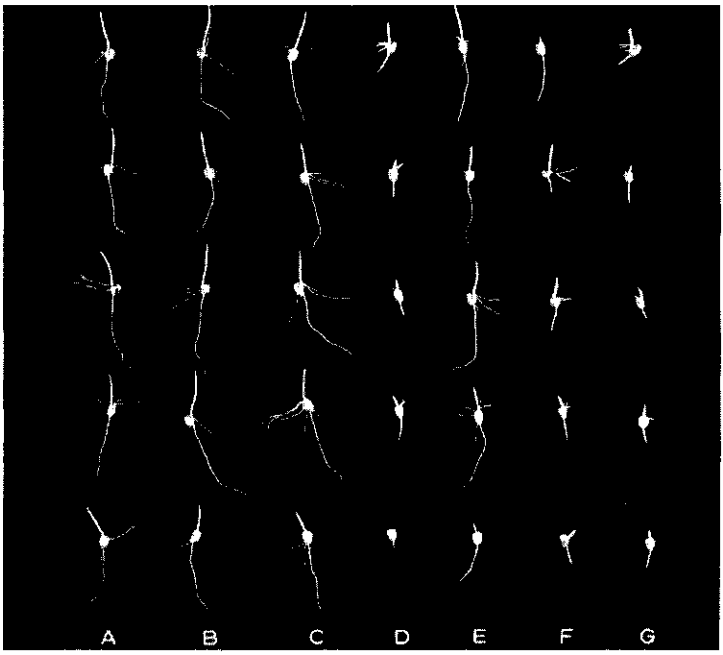

Figure 8. The effects of various seed dressings on early seedling growth of cv. Campo after 20 days at 10 °C and 3 days at 20 °C. For explanation see Table 21.

4.7.2 Results and discussion

Experiment 1. The effects of various seed dressings on shoot and root growth at temperatures of 10 °C and 20 °C.

The results for cv. Campo are given in Table 21 and Figure 8. At low temperature, shoot and root growth were strongly depressed by seed dressings D, F and G, containing lindane. At a temperature of 20 °C shoot growth was hardly affected, whereas root elongation tended to be reduced by lindane treatments. Differences between the other treatments were relatively small. Results for cv. Onix 95 are not presented; the data were indentical to those of cv. Campo. Seed germination, being 95 – 100 %, was not affected by the various seed dressings.

In the trial with four single-cross hybrids sown in pathogenic soil and germinated at 10 °C a similar effect of lindane was observed. Shoot length ratios F/B were 0.61, 0.66, 0.56 and 0.48 in SH5, SH7, SH8 and SH10, respectively; SH10 was most, and SH7 was least, affected. These data do not show a relationship with survival in the field experiment of 1975 (Table 3, S1 and S2).

It can be concluded that a 20-day exposure to 10 °C of seeds treated with dressings containing lindane results in a strong reduction of shoot and root growth.

Table 21. The effects of various seed dressings on shoot and root growth at low and high germination temperatures in cv. Campo (Experiment 1). Data are means of 40 seedlings; means within the columns followed by the same letter are not significantly different at $P \leqslant 0.05$.

	Seed dressing (% (w/w))			Length (mm/plant) of shoots and roots			
				20 d 10 °C, 3 d 20 °C		5 d 20 °C	
	thiram	methiocarb	lindane	shoot	root	shoot	root
A	–	–	–	36.0 d	66.8 c	23.1 a	59.4 cd
B	50 %	–	–	33.3 cd	64.4 c	28.8 bc	68.0 de
C	–	50 %	–	28.1 b	64.2 c	32.2 d	72.8 e
D	–	–	20 %	17.9 a	16.3 a	28.4 bc	38.4 a
E	25 %	25 %	–	29.9 bc	58.8 c	31.1 c	69.3 e
F	50 %	–	20 %	19.7 a	20.0 b	27.2 b	46.8 b
G	25 %	25 %	10 %	17.9 a	17.2 a	28.3 bc	52.8 c

Table 22. Effect of seed dressing with 50 % thiram (B) and 25 % thiram, 25 % methiocarp and 10 % lindane (G) on time of emergence, number of emerged plants and survival of four single-cross hybrids (Experiment 2). Seeds were sown in pathogenic soil. Treatment: 5 weeks 5 °C and 10 °C (alternating 4 d 5 °C and 3 d 10 °C), then 14 d 15 °C. Data of seed dressings B and G are means of 40 seeds. In addition, average data of sowing dates S1 and S2 of the field experiment described in Chapter 2 are presented for comparison; seed dressing in S1 and S2 was similar to that in G.

Geno-type	Time to emergence at 15 °C (days), $\bar{x} \pm s_{\bar{x}}$		Proportion of emerged plants (%)			Proportion of surviving plants (%)		
	B	G	B	G	S1, S2	B	G	S1, S2
SH4	9.2 ± 0.18	9.8 ± 0.22	85.0	50.0	51.2	90.0	65.0	72.5
SH5	8.1 ± 0.21	8.7 ± 0.20	75.0	62.5	23.8	75.0	75.0	28.8
SH7	8.2 ± 0.19	9.4 ± 0.25	62.5	22.5	20.0	65.0	37.5	60.0
SH9	10.9 ± 0.26	10.2 ± 0.19	67.5	50.0	86.2	87.5	62.5	91.2
Average	9.1	9.5	72.5	46.2		79.4	60.0	

Experiment 2. The effects of seed dressings with and without lindane on emergence and survival under cold-test conditions.

The results are given in Table 22. There were small differences in time of emergence for the two seed dressings. The percentage of emergence and survival, however, were lower for dressing G than B in all genotypes. This suggests an adverse effect of lindane in the early phase of seedling development.

The data for dressing G have been compared with data of the field experiment of

Table 23. The effect of seed dressing with 50 % thiram (B) and 50 % thiram and 20 % lindane (F) on time to emergence and shoot length (above soil) after emergence in three single-cross hybrids under field conditions (Experiment 3). Sowing date: 25 April 1978. Data are $\bar{x} \pm s_{\bar{x}}$ of 24 plants.

Genotype	Time from sowing to emergence (days)		Shoot length above soil (mm) at 17 days after sowing	
	B	F	B	F
SH2	13.8 ± 0.16	14.0 ± 0.21	25.3 ± 1.37	24.6 ± 1.88
SH9	14.5 ± 0.20	14.0 ± 0.20	21.2 ± 1.55	24.0 ± 1.53
SH10	14.6 ± 0.22	14.4 ± 0.16	19.5 ± 1.78	21.2 ± 1.22
Average	14.3	14.1	22.0	23.3

Chapter 2. There is no agreement between time of emergence for dressing G and the average time of emergence for sowing dates S1 and S2 (Table 4). The percentage of emergence shows some similarity for SH7 but not for SH5. The percentages of survival for dressing G and in the field experiment (i.e. percentage of emerged and germinated plants of Table 4) are completely different.

Experiment 3. The effects of a seed dressing containing lindane on emergence under normal field conditions.

Percentages of emergence were 97 % for dressing B and 100 % for F. Time of emergence and shoot length after emergence (Table 23) were similar for both seed dressings. The absence of adverse effects of lindane in this experiment may be attributed to the relatively high day time temperatures.

4.7.3 Conclusions

Seed dressings with 10 % or 20 % lindane have an adverse effect on root and shoot growth when seeds are germinated at 10 °C. At a germination temperature of 20 °C little or no effect could be detected. Seed dressings containing lindane reduce the number of emerging and surviving plants under cold-test conditions but genetic variation in this respect showed little agreement with the results of early sowing in the field.

4.8 Discussion and conclusions

This chapter describes experiments to determine low-temperature damage before emergence under laboratory conditions and investigate relationships with the data of the field experiment of Chapter 2.

Two types of chilling injury were found in the 10 single-cross hybrids (Table 15).

First, a 6-day exposure to 2 °C of seeds during imbibition reduced survival in some genotypes. Second, similar treatment of 3-day-old seedlings caused a reduction of shoot and root elongation, root necrosis and sometimes necrosis of the shoot. Necrosis of the roots was the most noticeable phenomenon that showed a wide genetic variation; it did not correlate with imbibitional chilling injury however.

There was little evidence for a relationship between chilling resistance (i.e. resistance to root necrosis) and survival or time to emergence under cold field conditions. Note that the chilling treatment of 2 °C for 6 days imposed an extreme stress that did not occur under the conditions of the 1975 field experiment. Reduced survival with early sowing in the field might have been caused by soil fungi and not by physiological damage. However results of a laboratory test (cold test) for resistance to soil fungi showed no clear relationship with survival in the field. Seeds were dressed with the fungicide thiram in the chilling experiments and the cold test. In the field experiment seeds were dressed with a mixture of thiram and the insecticides methiocarb and lindane. Lindane caused a significant reduction of shoot elongation in seedlings exposed to 10 °C (Figure 8). Lindane might have influenced emergence of the first sowing in the field.

A chilling treatment of seedlings before emergence had an adverse effect on seedling growth after emergence. Chilling sensivity increased with the developmental stage of the seedling (Tables 17 and 18). Various cold treatments above the chilling range had little effect on seedling growth after emergence, however, and there was no evidence of an adverse effect on seed reserves of such treatments (Table 19).

High correlations were found between chilling resistance of 3-day-old seedlings and shoot dry weight in the early sowings and the breeder's estimate of cold tolerance (Table 16). Whether or not there is a causal relationship remains uncertain.

5 The effects of temperature on seedling growth after emergence

5.1 Introduction

Shoot dry weight in the 6th-leaf stage was considered a major criterion for seedling growth at low temperature in the 1975 field experiments; large differences were found in the early sowings, S1, S2 and S3 (Table 5), and in the outdoor pot experiment (Table 8). Shoot dry weight in the 6th-leaf stage is the end result of growth processes before and after emergence. This chapter describes physiological and morphological characteristics of seedlings exposed to various temperature regimes after emergence.

A primary objective of the experiments described in this chapter was to investigate relationships between shoot dry weight in the 6th-leaf stage and various plant characteristics in a group of nine (of the ten) single-cross hybrids grown in pots of soil in growth cabinets at day/night temperatures of 15/10, 20/15 and 25/20 °C (Section 5.2). The shoot dry weight data showed, however, a poor correlation with those of the 1975 field experiment. Therefore it was attempted to reproduce field conditions by growing the hybrids in pots under various outdoor and greenhouse conditions (Section 5.3). Relationships between shoot dry weight data of those trials and the 1975 field trials are investigated and discussed.

Section 5.4 describes the time course of relative growth rate of the shoot and the time course of depletion of seed reserves from emergence until the 6th-leaf stage at low and intermediate temperature regimes for the single-cross hybrids SH2 and SH10 and cv. Fronica. In Section 5.5, a description is given of the investigation of the effect of temperatures in the range 7 – 21 °C on leaf elongation, relative growth rate of the shoot and leaf area in seedlings of SH2 and SH10 in the 3rd-leaf stage.

The significance of the results obtained in growth-room trials for seedling growth under cool field conditions and for selecting cold-tolerant genotypes is discussed in Sections 5.2, 5.3 and 5.6.

5.2 Growth characteristics of nine single-cross hybrids at day/night temperatures of 15/10, 20/15 and 25/20 °C

5.2.1 Introduction

The aim of this experiment was to investigate seedling growth after emergence under controlled conditions for three temperature regimes. Two growth characteristics were estimated: shoot elongation rate immediately after emergence, and elongation rate of

the 3rd leaf. At the 6th-leaf stage, shoot dry weight and various morphological characteristics were determined. Relationships between those characteristics, particularly relationships with shoot dry weight, were investigated.

5.2.2 Material and methods

Nine single-cross hybrids, i.e. genotypes SH2 – SH10, were used. Their seed was taken from the stock used in Chapter 2. Seeds were dressed with thiram 50 % (w/w) and sown at a depth of 5 cm in pots (diameter 16 cm) filled with potting compost. The pots were exposed to 20 °C for 7 days, from sowing to emergence. Thereafter the day/night temperature regimes used were: 15/10 °C for 35 days, 20/15 °C for 18 days and 25/20 °C for 11 days. Day/night temperatures of 15/10 °C was chosen for the lowest temperature regime as preliminary experiments had shown this treatment to be marginal for the growth of seedlings. Temperature treatments were carried out in growth cabinets. Other climate conditions were: day length 16 h; relative humidity 70 %; average light intensity (400 – 700 nm) 60 W m^{-2}, supplied by 24 fluorescent lights (Philips TL 33, 115 W) and three incandescent lights of 120 W. The cabinet had a horizontal air flow of 0.4 m s^{-1}. Light intensity in the corners of the cabinet was 12 – 15 % lower than in the centre. The cabinets had a square surface of 120 cm × 120 cm, which was divided into four small squares. On each small square one pot of each of the nine genotypes was placed in random order. To compensate for site effects of light and temperature the small-square groups were interchanged diagonally half way through the temperature treatment, so that pots from the centre were moved to the corners and vice versa. Each temperature treatment consisted of six growth-cabinet trials with four plants of each genotype.

Initial shoot-elongation rate was determined by measuring the distance between the tip of the coleoptile or the first leaf and soil level at the start of the temperature treatment and at 4, 2 and 1 days later for the treatments 15/10, 20/15 and 25/10 °C, respectively; shoot lengths at the second measurement were similar for the three temperature treatments.

Elongation rate of the 3rd leaf was determined by periodical measurement of the distance between leaf tip and soil level. Measurements were taken at intervals of 3 – 4 days from 7 to 25 days after the start of the 15/10 °C treatment; at intervals of 2 – 3 days from 5 to 14 days after the start of the 20/15 °C treatment; and at intervals of 1 day from 4 to 8 days after the start of the 25/20 °C treatment. The measurements showed that elongation rates were not constant, but increased from the appearance of the leaf, reached a maximum and then decreased (see also Figure 15). Data of the interval with the highest elongation rate of the individual plants were used to calculate the average elongation rates.

At the end of the temperature treatments, plants were harvested. Shoots were cut off at the coleoptilar node. Plant length was measured. Most of the plants were in the 6th-leaf stage (Figure 9). In the majority of the plants, the sheath of the 3rd leaf had become visible; in some plants even the sheath of the 4th leaf. The length of the sheath

Figure 9. Plants in the 6th-leaf stage of the 15/10 °C treatment, 35 days after emergence. Two plants on the left are of SH2. Two plants on the right are of SH10.

of the 3rd leaf was measured. The length and greatest width of the leaf blades were also measured. Leaf blades that had partly emerged from the whorl (Leaves 4, 5, 6 and 7) were cut off at the upperside of the highest visible leaf sheath. Leaf area (LA) was approximated by the formula:

$$LA = 0.75\,(l_1w_1 + l_2w_2 + l_3w_3 + l_4w_4) + 0.5\,(l_5w_5 + l_6w_6 + l_7w_7)$$

in which $l_1 - l_7$ and $w_1 - w_7$ are length and greatest width of the leaf blades or leaf blade parts. The coefficient 0.75 was adopted from Fakorede et al. (1977); for Leaves 5, 6 and 7 we used a coefficient of 0.5 because of the triangular shape of the leaf parts. Finally, dry weight of the total shoot was determined.

All measurements were carried out for individual plants.

5.2.3 Results and discussion

The results are presented in Tables 24 and 25. We will first consider the effects of temperature on average data of the whole group of genotypes. Figure 10 comprises temperature curves for initial shoot elongation rate and elongation rate of the 3rd leaf. It can be seen that leaf elongation is more depressed by low temperature than shoot elongation at emergence; the comparatively high value of initial shoot elongation at 15/10 °C may partly be attributed to an after-effect of the higher temperature before emergence (see also Section 5.4).

The effects of temperature on plant morphology are indicated in Table 25. In the 6th-leaf stage, the sheath and blade of the 3rd leaf were considerably longer for the higher temperatures, whereas the width of 3rd leaf was little affected. The size of the other leaf blades were similarly affected by temperature. Leaf area and shoot dry weight reflect the morphological effects of temperature (Table 24). The high values of leaf area and shoot dry weight at 20/15 °C and 25/20 °C can be attributed to etiola-

Table 24. Effect of day/night temperatures of 15/10, 20/15 and 25/20 °C on initial shoot elongation rate, elongation rate of the 3rd leaf, and leaf area and shoot dry weight at harvest (about 6th-leaf stage) in nine single-cross hybrids. From sowing to emergence plants were exposed to 20 °C for 7 days, from emergence to harvest to the above temperatures for 35, 18 and 11 days, respectively. Data are means of 6 trials with 4 plants.

Geno-type	Initial shoot elongation rate (mm/day)			Elongation rate of 3rd leaf (mm/day)			Leaf area in the 6th-leaf stage (dm^2/plant)			Shoot dry weight in the 6th-leaf stage (mg/plant)			Shoot dry weight ratio (15/10 °C)/(20/15 °C)
	15/10 °C	20/15 °C	25/20 °C	15/10 °C	20/15 °C	25/20 °C	15/10 °C	20/15 °C	25/20 °C	15/10 °C	20/15 °C	25/20 °C	
SH2	9.7	22.4	37.9	16.2	50.3	90.1	2.31	3.79	3.40	939	1645	1367	0.57
SH3	9.0	18.4	32.3	13.0	42.8	87.8	1.45	2.43	2.47	477	905	926	0.53
SH4	8.3	17.4	30.2	11.3	37.7	77.8	1.44	2.35	2.44	570	875	900	0.65
SH5	8.9	19.7	34.1	11.1	40.1	79.9	1.51	2.69	2.60	508	1074	996	0.47
SH6	11.0	23.5	41.2	11.4	37.4	71.9	1.94	3.43	3.00	482	1268	1184	0.38
SH7	11.5	22.3	40.2	13.5	44.0	85.6	1.94	3.55	3.44	523	1439	1353	0.36
SH8	10.2	20.7	40.2	14.2	47.8	96.1	2.17	3.70	3.40	652	1575	1387	0.41
SH9	10.5	20.7	36.4	13.6	44.4	86.9	1.92	3.64	3.68	500	1344	1361	0.37
SH10	11.0	21.3	37.4	11.8	38.6	76.0	1.90	3.31	3.25	578	1244	1272	0.46
Mean	10.0	20.7	36.7	12.9	42.6	83.6	1.84	3.21	3.08	581	1263	1194	0.47
Coefficient of variation	11.0	9.5	10.3	13.1	10.6	9.2	17.0	17.6	15.2	25.0	21.6	16.9	21.4

Table 25. Effect of day/night temperatures of 15/10, 20/15 and 25/20 °C on morphological characteristics of nine single-cross hybrids at about the 6th-leaf stage. From sowing to emergence plants were exposed to 20 °C for 7 days, from emergence to harvest to the above temperatures for 35, 18 and 11 days, respectively. Data are means of 6 trials with 4 plants.

Genotype	Length of the sheath of the 3rd leaf (mm)			Length of the 3rd leaf blade (mm)			Width of the 3rd leaf blade (mm)			Plant length (mm) (mm)		
	15/10 °C	20/15 °C	25/15 °C	15/10 °C	20/15 °C	25/15 °C	15/10 °C	20/15 °C	25/15 °C	15/10 °C	20/15 °C	25/15 °C
SH2	123	182	202	265	382	412	19.5	22.0	19.8	475	697	745
SH3	84	147	175	248	337	380	16.0	17.5	15.1	368	574	678
SH4	95	146	176	175	264	315	17.4	18.6	16.8	339	538	652
SH5	80	155	178	188	286	331	18.1	19.4	17.0	329	574	654
SH6	81	138	158	216	272	299	20.0	21.9	19.2	383	564	635
SH7	94	145	176	276	334	367	20.0	23.0	21.8	415	615	710
SH8	99	161	190	230	311	359	21.7	22.4	19.9	438	672	767
SH9	81	143	163	277	367	416	23.7	28.9	26.3	378	595	680
SH10	87	141	167	225	280	325	21.6	24.5	22.0	378	580	669
Mean	92	151	176	233	316	356	19.8	22.0	19.8	389	601	688
Coefficient of variation	14.9	9.0	7.6	15.7	13.3	11.7	12.0	15.5	17.0	11.9	8.7	6.5

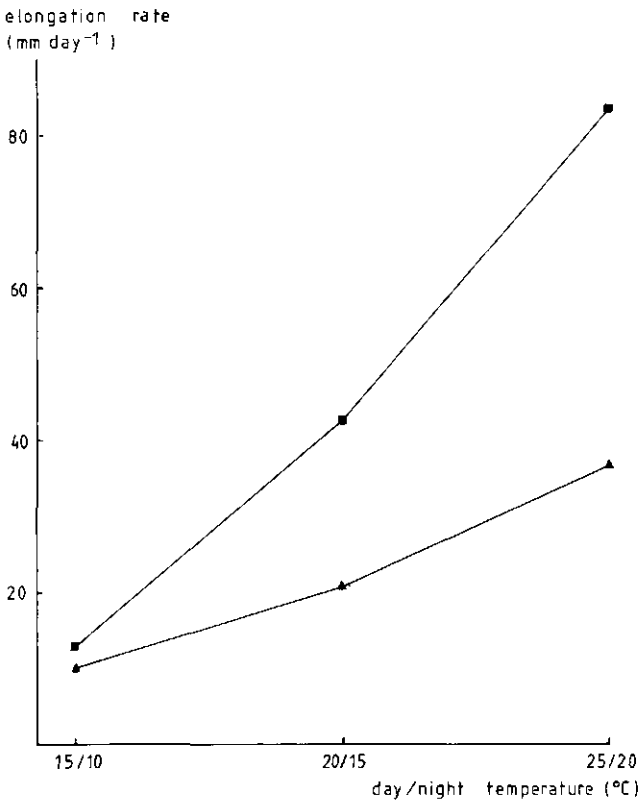

Figure 10. The relationship between temperature and initial shoot elongation rate (▲) and elongation rate of the 3rd leaf (■). Data are means of nine single-cross hybrids (data from Table 24).

tion. They are the result of too low a light intensity for those temperature regimes. The seedlings of the 15/10 °C treatment (Figure 9) approximate the shoot dry weight of seedlings in the 6th-leaf stage from field experiments (Tables 5 and 30) but they are more etiolated than field-grown seedlings (see also Figure 11). In some replicates at 15/10 °C seedlings of SH6, SH7, SH9 and SH10 became partly chlorotic.

The degree of genetic variation is indicated by the coefficient of variation (Tables 24 and 15). The highest variation was found for shoot dry weight for the 15/10 °C treatment. The genetic variation of most traits decreased with higher temperatures. The characteristics of growth and morphology are determined by genotype and temperature. Correlation coefficients for those characteristics between the temperature treatments are very high for most of the traits, particularly between 20/15 °C and 25/20 °C (Table 26). This implies little interaction between genotype and temperature. Shoot dry weight for 15/10 °C does not correlate significantly, however, with shoot dry weight of the 20/15 °C and 25/20 °C treatments.

Table 26. Correlation coefficients of growth and morphology characteristics between the three temperature treatments. Data from Tables 24 and 25. Levels of significance are $P \leqslant 0.05$ at $r = 0.666$ and $P \leqslant 0.01$ at $r = 0.798$.

Characteristic	15/10 °C vs. 20/15 °C	20/15 °C vs. 25/20 °C	15/10 °C vs. 25/20 °C
Initial shoot elongation rate	0.830	0.919	0.865
Elongation rate 3rd leaf	0.960	0.906	0.801
Length of the sheath 3rd leaf	0.850	0.944	0.873
Length of the blade 3rd leaf	0.881	0.978	0.815
Width of the blade 3rd leaf	0.953	0.990	0.931
Plant length	0.926	0.932	0.849
Leaf area	0.955	0.948	0.851
Shoot dry weight	0.621	0.942	0.424

Table 27. Correlation coefficients between shoot dry weight and growth and morphology characteristics for the three temperature treatments. Data from Tables 24 and 25. Correlation coefficients in brackets are calculated with data that does not include values for the genotype SH2.
* = $P \leqslant 0.05$ ** = $P \leqslant 0.01$.

	Shoot dry weight			
	15/10 °C		20/15 °C	25/20 °C
Initial shoot elongation rate	−0.094	(0.011)	0.753*	0.818**
Elongation rate 3rd leaf	0.754*	(0.280)	0.740*	0.423
Length of the sheath 3rd leaf	0.951**	(0.802*)	0.529	0.210
Plant length	0.765*	(0.455)	0.879**	0.647
Leaf area	0.655	(0.432)	0.956**	0.979**

Correlation coefficients between initial shoot elongation rate and elongation rate of the third leaf were 0.155, 0.231 and 0.121 at 15/10 °C, 20/15 °C and 25/20 °C, respectively. These low, non-significant values indicate that the growth characteristics are independent.

Shoot dry weight at the 6th-leaf stage is the most important criterion for seedling growth. Table 27 contains correlation coefficients between shoot dry weight and the other characteristics within the temperature treatments. Shoot dry weight correlates with initial shoot elongation for 20/15 °C and 25/20 °C treatments but not with those of the 15/10 °C treatment. Shoot dry weight correlates with elongation rate of the 3rd leaf for 15/10 °C and 20/15 °C but not significantly for 25/20 °C. High correlations were found between shoot dry weight and leaf area for 20/15 °C and 25/20 °C but not for 15/10 °C. A remarkably high correlation was found between the length of the 3rd

Table 28. Correlation coefficients between shoot dry weight in the 1975 field experiment and growth-room trials with nine single cross hybrids. Correlation coefficients in brackets are calculated with data that does not include values for the genotype SH2.
* = $P \leqslant 0.05$ ** = $P \leqslant 0.01$.

	Shoot dry weight growth-chamber experiment		
	15/10 °C	20/15 °C	25/20 °C
Shoot dry weight pot experiment (Table 8)	0.856** (0.172)	0.404	0.227
Shoot dry weight S3 field experiment (Table 5)	0.731* (0.029)	0.154	−0.149
Shoot dry weight S6 field experiment (Table 5)	0.151 (−0.290)	0.319	0.252

leaf sheath and shoot dry weight for the 15/10 °C treatment. Note that the correlations of shoot dry weight for 15/10 °C with the other characteristics are largely determined by the extreme values of SH2 since correlations without SH2 are much lower (values in brackets in Table 27). Therefore, the statistical relationships should be considered indicative of possible physiological relationships.

Correlation coefficients between shoot dry weight in the field experiments reported in Chapter 2 and the present growth-chamber trials are presented in Table 28. Shoot dry weight at 15/10 °C correlates with that of the outdoor pot experiment and to some extent with shoot dry weight of S3 in the field, but here again correlation coefficients are reduced to very low, non-significant levels when the data of SH2 are omitted. Shoot dry weight at 20/15 °C and 25/20 °C does not correlate with any of the field data; this may be due to the abnormal plant morphology in the growth chambers. A further discussion on the relationships between growth characteristics at 15/10 °C, 20/15 °C and 25/20 °C and data of other experiments is presented in Subsections 5.3.1 and 5.3.4 and Section 5.6.

5.3 Growth characteristics of nine single-cross hybrids grown in pots under field and greenhouse conditions

5.3.1 Introduction

The relatively low correlation between shoot dry weight at 15/10 °C and that of S3 in the field experiment (Table 28) may be caused partly by the effects of low temperature before emergence in S3. Also plants in pots with potting compost grow faster than in soil (Chapter 2). Plant growth in the field might, therefore, have been restricted by adverse soil conditions like soil structure and fertility or by the activity of soil fungi. The absence of correlations between shoot dry weight at 20/15 °C or 25/20 °C and that of S6 in the field experiment (Table 28) may be due to differences in light intensity and temperature, but also to differences in soil conditions.

An experiment was done to reproduce the effects of temperature under field conditions, but without any adverse effects of soil. To ensure that, plants were grown in pots with potting compost. The effects of low, moderate and high temperature were studied by growing the plants outdoors at two sowing dates and in a greenhouse. The effects of low temperature before emergence were investigated by comparing plants grown outdoors with those kept in a greenhouse from sowing to emergence. After emergence both groups were exposed to the same field conditions.

5.3.2 Materials and methods

The experiment was carried out in 1977. The materials and techniques used are similar to those described in Subsection 5.2.2. Four treatments were given:
Treatment A – Low temperature before emergence and low temperature after emergence
Treatment B – High temperature before emergence and low temperature after emergence
Treatment C – Moderate temperature before emergence and moderate temperature after emergence
Treatment D – High temperature before emergence and high temperature after emergence.

Low temperature in Treatment A was attained by exposing the pots to field conditions after early sowing, moderate temperature in Treatment C by exposing them to field conditions after late sowing, and high temperature in Treatment D by exposing them to greenhouse conditions. In Treatment B a high temperature was given from sowing to emergence by exposing the pots to greenhouse conditions; thereafter the pots were exposed to field conditions. Time of emergence in Treatments A and B were similar, so that the growth period after emergence was subject to the same field conditions. Details on dates of sowing, emergence and harvest, duration of growth periods and average temperatures are given in Table 29.

Time of emergence was recorded in Treatments A and C, initial shoot length in Treatments A and B on 11 May, and shoot dry weight in the 6th-leaf stage for all four treatments on the harvest dates indicated in Table 29.

Characteristics of shoot morphology of the genotypes SH2 and SH10 were determined on the dates of harvest.

5.3.3 Results and discussion

First, consider the effects of low temperature before emergence by comparing the effects of Treatments A and B (Table 30). On 11 May, 98 % of the plants of both groups had emerged, which indicates that low temperature before emergence in Treatment A had not affected survival. Variation in the initial shoot length in Treatment A did not correlate with that in Treatment B. The initial shoot length ratio Treatment A/Treatment B, an indicator of resistance to low temperature before emergence,

Table 29. Schedule of the treatments. The date of shoot length measurements, i.e. when the shoots were 2 – 3 cm above soil level, was considered as date of emergence.

Treatment	Sowing date	Emergence date	Harvest date	Conditions from sowing to emergence			Conditions from emergence to harvest		
				place	duration (days)	mean air temperature (°C)	place	duration (days)	mean air temperature (°C)
A	13 April	11 May	3 June	field	28	8.6	field	23	13.0
B	4 May	11 May	3 June	greenhouse	7	20.0	field	23	13.0
C	18 May	29 May	17 June	field	11	14.8	field	19	14.4
D	18 May	24 May	6 June	greenhouse	6	22.8	greenhouse	13	20.2

Table 30. Time to emergence, initial shoot length in Treatments A and B and shoot dry weight at the 6th-leaf stage of nine single-cross hybrids grown at four temperature treatments under field and greenhouse conditions. Data are means of 24 plants. The schedule of treatments is given in Table 29.

Genotype	Time to emergence (days)		Initial shoot length on 11 May (mm)		Shoot dry weight (mg/plant)				Initial shoot length ratio	Shoot dry weight ratio	Shoot dry weight ratio
	A	C	A	B	A	B	C	D	A/B	A/B	A/D
SH2	22.1	9.5	40.3	32.6	539	645	654	903	1.24	0.84	0.60
SH3	24.1	10.1	26.4	30.8	346	511	436	685	0.86	0.68	0.51
SH4	24.1	10.0	24.8	28.9	388	502	461	523	0.86	0.77	0.74
SH5	23.4	9.6	27.8	35.1	367	521	469	669	0.79	0.70	0.55
SH6	23.4	10.0	42.2	24.8	441	481	551	850	1.70	0.92	0.52
SH7	22.3	10.0	45.3	29.8	403	477	495	750	1.52	0.84	0.54
SH8	22.4	9.4	37.3	30.6	435	461	570	891	1.22	0.94	0.49
SH9	23.7	9.8	34.3	36.8	437	516	544	919	0.93	0.85	0.48
SH10	22.9	9.8	36.5	28.5	492	591	569	951	1.28	0.83	0.52
Mean	23.2	9.8	35.0	30.9	428	523	528	794	1.16	0.82	0.55
Coefficient of variation	3.4	2.6	20.8	11.6	14.2	11.3	13.0	18.3	27.6	10.8	14.5

Table 31. Correlations between three estimates of adverse after-effects of low temperature before emergence * = $P \leqslant 0.05$ ** = $P \leqslant 0.01$.

	1	2	3
1 Initial shoot length ratio A/B (Table 30)	1.000		
2 Shoot dry weight ratio A/B (Table 30)	0.733*	1.000	
3 Shoot dry weight ratio ½ (S1 + S2)/S3 (Table 5)	0.857**	0.748*	1.000

Table 32. The effect of various temperature treatments on morphological characteristics of the single-cross hybrids SH2 and SH10 in the 6th-leaf stage. Data are means of 24 plants.

Treatments	Sheath length 3rd leaf (mm)		3rd leaf blade				Plant length (mm)	
			length (mm)		width (mm)			
	SH2	SH10	SH2	SH10	SH2	SH10	SH2	SH10
I Experiments Section 5.2								
A 20 °C – 15/10 °C	123	87	265	226	19.5	21.6	475	378
B 20 °C – 20/15 °C	182	141	382	289	22.0	24.5	697	580
C 20 °C – 25/20 °C	202	169	412	325	19.8	22.0	745	669
II Experiments Section 5.3								
A 8.6 °C – 13.0 °C	67	55	160	160	19.0	20.0	253	235
B 20.0 °C – 13.0 °C	70	54	181	160	21.1	23.2	274	238
C 14.8 °C – 14.4 °C	90	70	190	162	22.3	22.6	332	287
D 22.8 °C – 20.2 °C	146	116	303	240	21.0	22.7	548	498

showed considerable genetic variation. Just after emergence (11 May) shoots of Treatment A were on average a little longer than those of Treatment B. Shoot dry weight in the 6th-leaf stage was, however, higher in Treatment B than in Treatment A. This implies that low temperature before emergence had an adverse after-effect on shoot growth. The shoot dry weight ratio Treatment A/Treatment B is a measure of this after-effect. The question arises whether correlations exist between initial shoot length ratio Treatment A/Treatment B and shoot dry weight ratio Treatment A/Treatment B, and also whether these two indicators correlate with the after-effects of early sowing in the 1975 field experiment, i.e. with the shoot dry weight ratio ½(S1 + S2)/S3 (Table 5). Table 31 shows significant correlations between the three estimates. This suggests that adverse effects of low temperature before emergence influenced final seedling weight.

Second, compare times of emergence of early sowing (Treatment A) and late sowing (Treatment B) with those of S3 and S6 of the 1975 field experiment (Table 4). There is a high correlation between times of emergence in Treatment A and S3 (r =

Table 33. Correlation coefficients between shoot dry weight of the field and greenhouse trials of 1977 (Table 30) and shoot dry weight of the field experiment of 1975 (Table 5 and 8) and growth-chamber experiments (Table 24) of nine single-cross hybrids. Correlation coefficients in brackets are calculated with data that does not include values for the genotype SH2. * = $P \leqslant 0.05$ ** = $P \leqslant 0.01$.

Treatments 1977	Field experiment 1975				Growth-chamber experiments			
	S3	S6	pot experiment		15/10 °C		20/15 °C	25/20 °C
A 13 April Field	0.322	0.356	0.725*	(0.334)	0.750*	(0.408)	0.726*	0.704*
B 4 May Greenhouse - Field	0.517	0.203	0.842**	(0.526)	0.700*	(−0.067)	0.232	0.170
C 18 May Field	0.363	0.433	0.682*	(0.197)	0.763*	(0.452)	0.842**	0.781*
D 18 May Greenhouse	−0.092	0.568	0.360	(0.250)	0.330	(0.188)	0.756*	0.844**

Table 34. Correlation coefficients between shoot dry weight ratios at low/high growth temperature of three experiments. Correlation coefficients in brackets are calculated with data that does not include values for the genotype SH2. * = $P \leqslant 0.05$ ** = $P \leqslant 0.01$.

	1	2	3
1 Field 1975, S3/S6 (Table 5)	1.000		
2 Field and greenhouse 1977, A/D (Table 30)	0.874** (0.813*)	1.000	
3 Growth room 15/10 °C / 20/15 °C (Table 24)	0.843** (0.888**)	0.810** (0.810**)	1.000

5.4 Time course of seed reserves and RGR of the shoot at 15/10, 16/10 and 20/15 °C

5.4.1 Introduction

In the experiments reported in Chapter 2 and Sections 5.2 and 5.3, the effects of temperature on seedling growth were estimated by determining shoot dry weight in the 6th-leaf stage. In the field experiment described in Chapter 2, shoot size in the 3rd-leaf stage did not correlate with shoot weight in the 6th-leaf stage (Table 7). The growth-room experiments showed that shoot elongation rate at emergence did not correlate with elongation rate of the 3rd leaf (Section 5.2.3). These data show that the genotypic variation in shoot growth rate is dissimilar for various stages of seedling development. Quantitative data on shoot dry matter accumulation during those stages are required for a better understanding of the dissimilar growth rates. This section describes investigations into RGR of the shoot (RSGR) at five time intervals from emergence to the 6th-leaf stage in two single-cross hybrids, SH2 and SH10, and cv. Fronica kept in growth cabinets under low and intermediate temperature regimes. Decrease of dry matter of the seed was also determined since depletion of seed reserves may lead to a decrease in seedling growth (Cooper & MacDonald, 1970).

5.4.2 Material and methods

The experiments were carried out with the cold-resistant genotype SH2, the cold-sensitive SH10 and the cultivar Fronica. Seeds of SH2 and SH10 used were taken from a new stock produced in a greenhouse in 1976.

The experimental conditions were the same as described in Subsection 5.2.2. Six seeds were sown in pots that were exposed to 20 °C for 7 days, from sowing to emergence. Two temperature regimes were tested, 15/10 °C and 20/15 °C. Plants were harvested six times, at intervals of 7 days for 15/10 °C and 3 – 4 days for 20/15 °C. Growth of SH2 and SH10 was tested in one growth cabinet containing 18 pots of each genotype. Growth of cv. Fronica was tested in another growth cabinet containing 36 pots. When sampling, one plant per pot was taken for each genotype. Dry weight of the shoots (cut off at the coleoptilar node) and the seeds was determined for each plant. The parts of the seed included in the values of the dry weight are endosperm, seed coat, scutellum, and the embryonic axis between the base of the mesocotyl and the primary root. For each genotype, the total dry weight of the seeds at sowing was equal for each sampling time; mean dry weight of the seeds was 186 mg for SH2, 356 mg for SH10, and 298 mg for cv. Fronica. RSGR for the five time intervals was calculated from the mean data of shoot dry weight at the six sampling times.

At 15/10 °C, some chlorosis developed in cv. Fronica. RSGR in all three genotypes was highest during the first period (Figure 13). To investigate whether this was an after-effect of the high temperature of the preceding period, an additional experiment was done with cv. Fronica. In this experiment one temperature regime, 16/10 °C, was

used (day length 16 h) for the whole growth period; 16 °C was chosen instead of 15 °C to avoid chlorosis.

5.4.3 Results and discussion

Figure 12 illustrates the six stages of seedling development at which shoot dry weight and seed dry weight were determined, in this case at 15/10 °C. The results of the 15/10 °C trials with SH2 and SH10 are presented in Figure 13A. Twenty-eight days after sowing, i.e. 21 days after starting the temperature treatments, seed reserves are nearly depleted. The remaining seed weight is made up of the embryonic axis, scutellum and seed coat. RSGR was about two times higher during the first week, i.e. until about the end 3rd-leaf stage, than during the rest of the growth period. In SH2, RSGR is about constant after the first week, but in SH10 the decline of RSGR continued, reaching a very low value in the third week, followed by a recovery in the fourth and fifth week. In cv. Fronica (Figure 13B) the lowest RSGR was in the fourth week. Exposure of cv. Fronica to a low temperature (16/10 °C) from sowing (Figure 13C) resulted in a similar time course of RSGR. The high initial values of RSGR in the 15/10 °C trials, therefore, are not an after-effect of the 20 °C treatment before emergence. The lowest value of RSGR coincides with depletion of seed reserves and may be associated with the transition of shoot growth on seed reserves to supply of carbohydrates produced by photosynthesis only. The plants of SH10 were slightly chlorotic, whereas the plants of SH2 were green. The extremely low RSGR after seed depletion in SH10 is probably due to impairment of the photosynthetic apparatus. During the

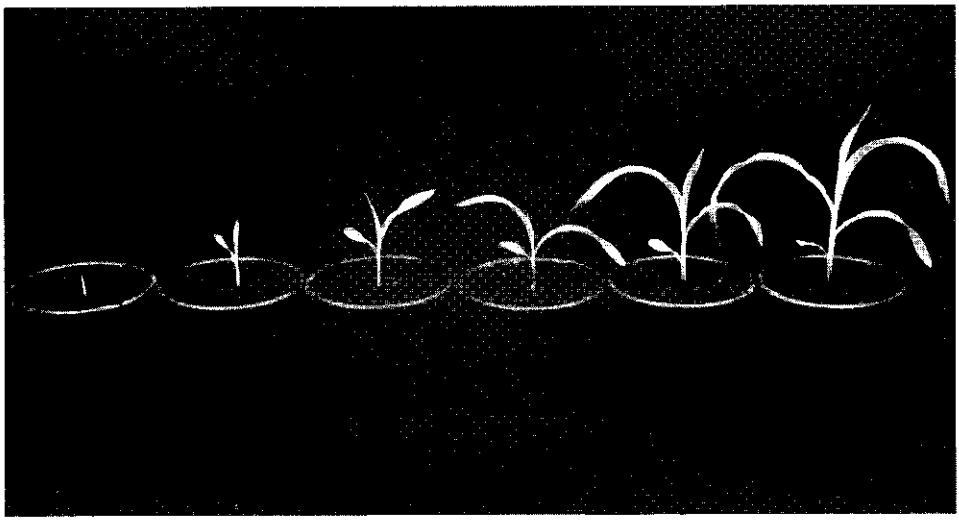

Figure 12. The six stages of seedling development at which shoot and seed dry weight were determined; seedlings shown are SH10 grown at 15/10 °C.

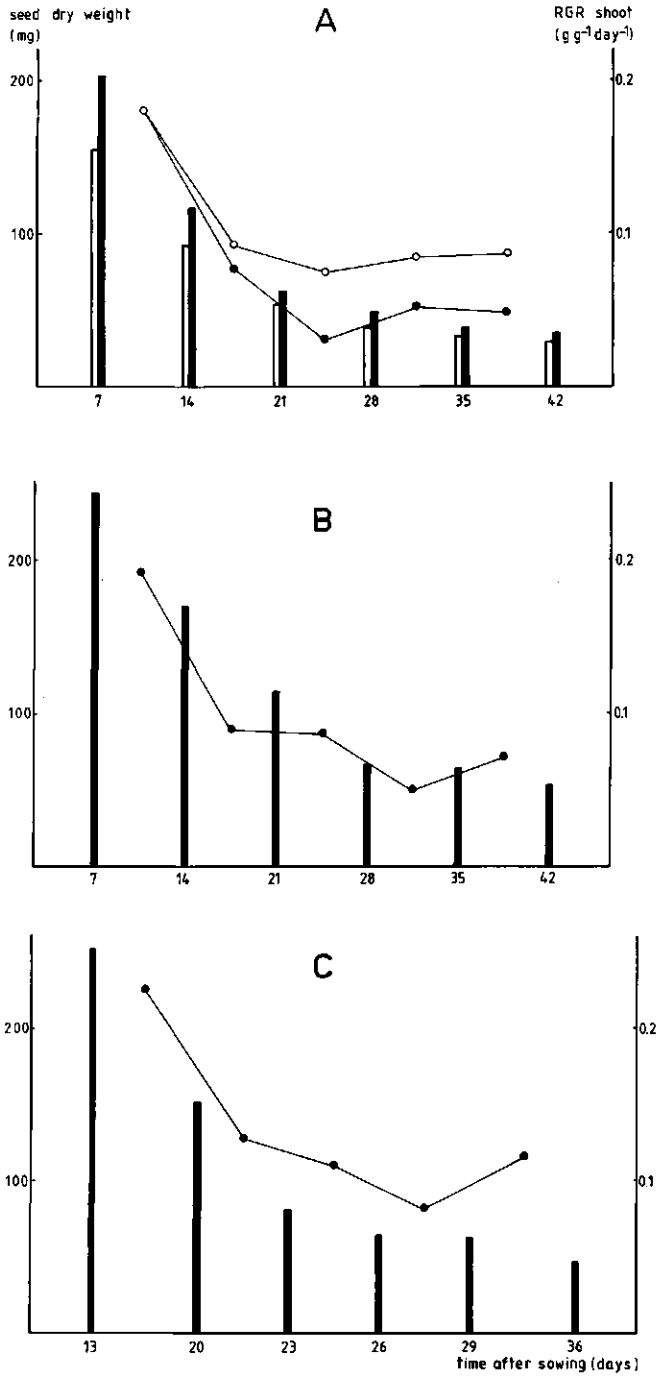

Figure 13. Course of RSGR (circles) and seed dry weight (columns) from emergence until about the 6th-leaf stage at low temperatures. A: SH2 (open circles and columns) and SH10 (closed circles and columns), 15/10 °C. B: cv. Fronica, 15/10 °C. C: cv. Fronica, 16/10 °C.

first week, i.e. from emergence until the 3rd-leaf stage, RSGR of both hybrids were similar. The final shoot dry weight of SH2 was 405 mg; for SH10 it was 180 mg. This difference is due to the different RSGR after the 3rd-leaf stage (Figure 13A).

At 20/15 °C, RSGR was much higher than at 15/10 °C for all genotypes. During the first period after emergence RSGR at 20/15 °C was higher than during the rest of the time, particularly in SH10, but the decline after that period was less pronounced than at 15/10 °C (Figure 14). No extremely low growth rates were found, which may indicate that the transition to photosynthetic supply of carbohydrates was not critical. However Bourdu & Gregory (1983) showed that even at 25/22 °C a crisis in growth occurred in several maize varieties at 9 – 10 days after sowing in a similar stage of leaf development and seed-reserve depletion as in the above experiments. This growth crisis coincided with a transient arrest of seed depletion and was attributed to the tran-

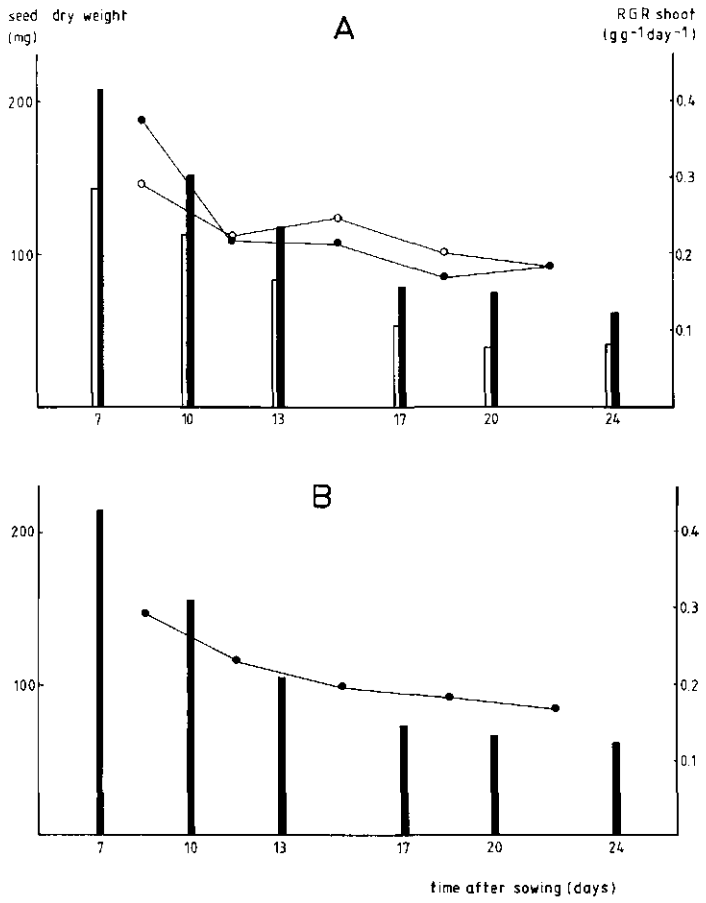

Figure 14. Course of RSGR (circles) and seed dry weight (columns) from emergence until about the 6th-leaf stage at moderate temperatures. A: SH2 (open circles and columns) and SH10 (closed circles and columns). B: cv. Fronica, 20/15 °C.

sition of shoot growth to autotrophic carbon supply (Deléens et al., 1984). The crisis was of very short duration, about one day, and it may have been overlooked in our 20/15 °C experiments.

It can be concluded that the relative growth rate of the shoot at 15/10 or 16/10 °C was much higher from emergence until about the 3rd-leaf stage than after that period. At 20/15 °C the decline of RSGR was much smaller than under the lower temperature regimes. An exact physiological explanation for those findings and their significance for breeding of cold-tolerant maize genotypes deserve further investigation.

5.5 The effects of low temperature on leaf extension and shoot dry matter accumulation in SH2 and SH10

5.5.1 Introduction

Miedema & Sinnaeve (1980) demonstrated a relatively high rate of photosynthesis in maize seedlings at air temperatures of 10 °C. They suggested that leaf extension rather than photosynthesis is limiting growth at low temperatures. The following experiment was done to investigate the extension rate of the 3rd leaf and relative growth rate of the shoot at temperatures around and above the minimum temperature for growth in a cold-resistant (SH2) and a cold-sensitive (SH10) genotype; these two correspond with the genotypes F and D in the study on photosynthesis by Miedema & Sinnaeve (1980).

5.5.2 Materials and methods

Two experiments were carried out.

Experiment 1. Plants of the single-cross hybrids SH2 and SH10 were subjected to five constant temperature regimes in the range 7 – 21 °C. Seeds were produced in a greenhouse in 1976. Plants were raised out of doors under moderate temperature conditions until the 3rd leaf became visible. From 170 plants of each genotype raised, seven uniform groups of 16 plants were selected. Two groups (32 plants) were used to estimate initial leaf area and shoot dry weight. The other groups were transferred to growth cabinets and subjected to five temperature treatments (Table 35). Other climatic conditions and techniques are the same as described in Subsection 5.2.2.

At the end of the temperature treatments, leaf area, shoot dry weight and dry matter content were determined; severely injured and dead plants were omitted. Relative growth rates of leaf area (RLAGR) and shoot dry matter (RSGR) were calculated from the initial and final data.

Experiment 2. A second experiment was conducted because of some technical problems of Experiment 1. First, in the low temperature treatments (7, 10, 11.5 °C) soil temperatures at 2 cm below soil surface were 0.5 – 1.0 °C higher than air temperatures

during the light period. Second, leaf extension rate decreased during these temperature treatments because of some kind of low temperature damage. For these reasons the low temperature treatments were repeated, but with the following modifications. The pots were covered with aluminium foil, which prevented warming of the soil. The growth period was shortened to 8 days. The experiment was carried out with SH10 material only. Temperature treatments were 7, 10 and 13 °C. Response to temperature was tested among seedlings in a 3rd-leaf stage and in a second group raised to the 5th-leaf stage, to investigate the effect of seedling stage. For each seedling stage, 18 plants were used for each temperature treatment.

5.5.3 Results and discussion

Experiment 1. Figure 15 shows the elongation rate of the 3rd leaf of SH10. At 21 °C, and to some extent at 16 °C, the leaf elongation rate changed with leaf development; it increased, reached a maximum and then decreased. At lower temperatures (data of Exp. 1 not presented in Figure 15) the leaf elongation rate only decreased. At 7 °C, leaf elongation in SH2 decreased from 1.9 to 0.7 mm h^{-1}, and in SH10 from 2.3 to 0.5 mm h^{-1} during exposure. A smaller decrease was found at 10 and 11.5 °C. Pro-

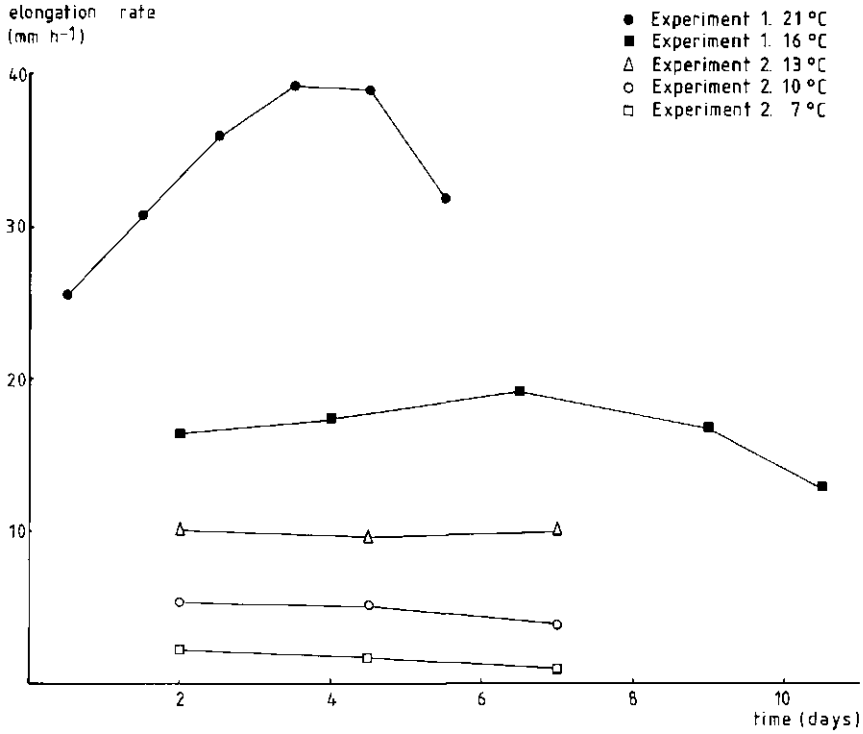

Figure 15. Course of the elongation rate of the 3rd leaf of SH10 at constant temperatures of 7-21 °C.

Table 35. Experiment 1. The effects of temperature on leaf growth, shoot growth and shoot dry matter percentage in seedlings of SH2 and SH10. Leaf elongation rates are mean values from the 2nd to 5th day of the temperature treatment. Data of RLAGR and shoot dry matter percentage at 7 °C are omitted because of a high proportion of wilted plants.

Treatment	Elongation rate 3rd leaf (mm d^{-1}), $\bar{x} \pm s_{\bar{x}}$		RLAGR ($cm^2\ cm^{-2}\ d^{-1}$)		RSGR ($g\ g^{-1}\ d^{-1}$)		Shoot dry matter content (%)	
	SH2	SH10	SH2	SH10	SH2	SH10	SH2	SH10
7 °C, 16 days	1.9 ± 0.08	2.3 ± 0.13			0.022	0.019		
10 °C, 22 days	3.7 ± 0.13	4.2 ± 0.10	0.038	0.040	0.039	0.032	9.5	8.9
11.5 °C, 23 days	6.3 ± 0.13	6.0 ± 0.18	0.052	0.050	0.050	0.040	8.3	8.2
16 °C, 11 days	18.0 ± 0.24	16.8 ± 0.28	0.154	0.160	0.146	0.139	7.8	8.3
21 °C, 6 days	41.6 ± 0.75	36.2 ± 0.46	0.276	0.278	0.247	0.231	7.5	8.2

longed exposure to low temperature resulted in visible injury. Leaves of some plants became partly necrotic, other plants began to wilt. These injuries appeared after about one week at 7 °C and after two weeks at 10 and 11.5 °C. At the end of the exposure some of the wilted or dead plants had necrotic shoot meristems. SH10 seemed to be more sensitive to this injury and to leaf growth inhibition than SH2.

At 7 – 11.5 °C, all newly formed leaf tissue was chlorotic. The proportion of chlorotic leaf area increased with temperature; it was about 70 % of the total leaf area at 11.5 °C. The degree of chlorosis was similar for both genotypes, although in other experiments with less-severe temperature stress (Table 6) SH10 was more sensitive to chlorosis than SH2. At 16 °C and 21 °C no chlorosis was observed.

Table 35 gives data of leaf elongation, RLAGR and RSGR. The leaf elongation rates are of the first period of exposure to diminish the effects of low temperature damage. All growth parameters were very low for the temperature range 7 – 11.5 °C, they increased strongly at higher temperatures. Shoot dry matter content at the end of exposure tended to be higher at lower temperatures.

The two genotypes showed little difference in low temperature response in this experiment; their final shoot dry weights were similar within each temperature treatment.

Experiment 2. Exposure of seedlings to 7 °C caused a steady decrease in the elongation rate of the 3rd leaf (Figure 15); at 10 °C a similar decrease began after five days of exposure. In contrast, at 13 °C the leaf growth rate was constant during the exposure time. Seedlings exposed to low temperature at a later stage of development showed a similar pattern in the elongation rate of their 5th leaf. At 7 °C, leaf margins and tips became necrotic and some seedlings began to wilt at the end of the exposure period; this injury was observed in both seedling stages. Those data show that low temperature damage does not depend on the stage of seedling development.

Table 36. Experiment 2. The effects of low temperature on leaf growth and RSGR in seedlings of SH10 that were in the 3rd-leaf stage at the start of the temperature treatments. In addition, elongation rate of the 5th leaf is presented of seedlings exposed to low temperature in the 5th-leaf stage. All data are based on a 8-day exposure time. Values in brackets are percentages of the data at 13 °C.

Temperature	Seedlings 3rd-leaf stage			Seedlings 5th-leaf stage
	RSGR ($g\ g^{-1}\ d^{-1}$)	RLAGR ($cm^2\ cm^{-2}\ d^{-1}$)	Elongation rate 3rd leaf ($mm\ d^{-1}$)	Elongation rate 5th-leaf ($mm\ d^{-1}$)
7 °C	0.019 (17)	0.024 (20)	1.57 (16)	2.84 (18)
10 °C	0.060 (53)	0.058 (48)	4.80 (48)	8.87 (57)
13 °C	0.114 (100)	0.122 (100)	9.90 (100)	15.43 (100)

Again newly formed leaf tissue was chlorotic at low temperatures (7 – 13 °C). At 7, 10 and 13 °C the proportion of new, chlorotic leaf area was about 17, 37 and 62 %, respectively.

Table 36 shows that RGR of the shoot and leaf area, and elongation rate of the 3rd leaf similarly decreased as the temperature was lowered from 13 °C to 7 °C. At all three temperatures the elongation rate of the 5th leaf was higher than that of the 3rd leaf. This is attributed to differences in leaf size. The relative decrease of leaf elongation with decrease in temperature showed little differences between the leaves.

From the two experiments it can be concluded that the minimum temperature for leaf growth and dry matter accumulation is less than 7 °C, but long-term exposure to temperatures of 10 °C or lower results in physiological damage and eventually the death of the plants (see also Chapter 6).

5.6 Discussion and conclusions

There are two aspects to the experimental data presented in this chapter. One aspect is the effects of low temperature on the physiology and morphology of seedlings observed in all genotypes investigated. The other is the genotypic variation in response to low temperature and its significance for the selection of cold-tolerant maize genotypes. For both aspects the relationship between data obtained under controlled conditions and the results of field trials will be considered.

5.6.1 Physiology and morphology of seedling growth at low temperature

One of the most remarkable results was that for low temperature regimes (15/10 and 16/10 °C) RSGR is not constant (Figure 13); the shoots grow much faster from emergence until about the 3rd-leaf stage than from the 3rd to the 6th-leaf stage. For a

regime 20/15 °C the decline in RSGR was much smaller (Figure 14). Initial shoot growth is less sensitive to low temperature than shoot growth or leaf extension in later stages of seedling development (Figure 10). The decline of RSGR at low temperature coincides with depletion of seed reserves. This suggests that the decrease of shoot growth is caused by reduced supply of carbohydrates, which may be associated with cold-induced damage to the photosynthetic apparatus other than chlorosis (Section 9.3). Further research is needed to investigate this possibility.

In the above experiments on RSGR, described in Section 5.4, the plants were exposed to low temperature after emergence or sowing. In the experiments described in Section 5.5, seedlings were reared under non-stress conditions until the 3rd leaf appeared and then exposed to constant temperatures ranging from 7 to 21 °C. At 7 °C and 10 °C the elongation rate of the 3rd leaf decreased, due to some kind of physiological damage, with time of exposure. At 13 °C the leaf elongation rate was constant over a period of 7 days, whereas at higher temperatures it increased with leaf development. With a lowering of temperature from 13 °C to 7 °C the decrease of leaf elongation rate was similar to the decrease of RSGR (Table 36). It seems unlikely that those effects of low temperature were due to reduced carbohydrate supply because, first, seed reserves were not yet depleted in this stage of seedling development, and second, in non-damaged plants, photosynthesis in less reduced by low temperature than is leaf elongation (Miedema & Sinnaeve, 1980; see also Miedema, 1982, Figure 2). We may assume, therefore, that growth reduction by short-term exposure to low temperature is due to restrained leaf growth. Long-term exposure to low temperature causes several types of physiological damage including damage to the photosynthetic apparatus.

Low temperature also affected seedling morphology. At similar stages of leaf appearance, seedlings grown in growth cabinets at 15/10 °C were much shorter than those grown at 20/15 °C or 25/20 °C (Table 32). The etiolation at high temperature is not only caused by high temperature, however, but also by the relatively low light intensities in the growth cabinets (Warrington et al., 1978).

5.6.2 Genotypic variation

A major objective of the experiments described in this chapter was to find relationships between seedling growth under cold field conditions and growth characteristics under controlled conditions. Such relationships may be useful for the development of screening techniques for cold tolerance.

The first question to be answered is whether genotypic variation found in the field could be reproduced in growth chambers. Shoot dry weight of nine single-cross hybrids in the 15/10 °C growth-chamber trial correlated with shoot dry weight of S3 and the outdoor pot experiment of 1975, and also with shoot dry weight of the 1977 outdoor pot experiment (Tables 28 and 33). However, no correlations were found when the data of SH2 were omitted from the calculations. SH2 has a high degree of cold tolerance and its shoot dry weight was much higher than that of the other hybrids. The

However, no quantitative data are available on the influence of the degree of chlorosis on seedling growth under field conditions. Therefore attempts were made to define chlorosis-inducing conditions and to assess the effects of various degrees of chlorosis on leaf extension and shoot dry matter accumulation in genotypes differing in sensitivity to chlorosis (Sections 6.2 – 6.5).

6.1.2 Chilling injury

The experiments reported in Chapter 4 showed that before emergence seedlings are sensitive to chilling temperatures of 2 – 4 °C. Interesting differences between genotypes were found. Section 6.6 describes the effects of similar but less severe chilling treatments on seedlings after emergence. The main aim of these experiments was to assess visible damage in different genotypes and to investigate possible relationships between sensitivity to chilling before and after emergence, and between sensitivity to chilling after emergence and sensitivity to chlorosis.

6.2 Chlorosis and leaf extension in ten single-cross hybrids

A preliminary experiment was done to investigate the relationship between chlorosis and leaf extension. Plants of the 10 single-cross hybrids (8 plants of each genotype) were reared in a greenhouse at 20 °C until the 3rd leaf appeared. Then the plants were transferred to a greenhouse and exposed to an air temperature of 10 °C and natural light for the period 22 February – 7 March. The extension rate of the 3rd leaf was measured. The average extension rate was 3.4 mm day^{-1}, ranging from 2.9 mm day^{-1} in SH5 and SH8 to 4.3 mm day^{-1} in SH7. At the end of the period newly formed leaf parts were more or less chlorotic. Some of the most chlorotic plants also showed symptoms of leaf necrosis.

The degree of chlorosis differed within the genotypes. This was attributed to site effects. Clear differences in chlorosis were also observed between the genotypes. A visual estimate of resistance to chlorosis in this experiment correlated ($r = 0.845^{**}$) with the estimate in the 1975 field experiment, reported in Chapter 2. The correlation between resistance to chlorosis and leaf extension was $r = -0.638^{*}$. Although this correlation was low, its negative value may indicate that chlorosis increases with leaf extension.

After their exposure to 10 °C the plants were transferred to a greenhouse with a temperature of about 20 °C. Most of the chlorotic leaf area turned green within 3 days, but severely chlorotic leaves did not recover.

Although this experiment was done with a small number of plants, it can be concluded that in a greenhouse at an air temperature of 10 °C genotypes can be screened for resistance to chlorosis. The negative correlation between resistance to chlorosis and leaf extension at low temperature deserves further investigation (see also Chapter 8).

6.3 The effects of light intensity during cold treatment on chlorosis

6.3.1 Introduction

As described in the preceding section, Section 6.2, chlorotic or partly chlorotic plants can be obtained by growing seedlings in a greenhouse at an air temperature of 10 °C. Genotypes differing in sensitivity to chlorosis can be used to investigate the effects of chlorosis on plant growth. However, genotypic variation in growth rate may be due to other factors as well. A better approach is, therefore, to compare the growth rate of chlorotic and non-chlorotic plants within a genotype. Such plants can be obtained by growing them at different temperatures, but then various temperature-dependent processes may be involved. To avoid this complication we tried to obtain chlorotic and non-chlorotic plants by varying the light intensity in a greenhouse with an air temperature of 10 °C. The main aim of the experiment was to find out whether in this way different degrees of chlorosis could be obtained. In addition shoot dry weight of the plants was estimated after exposure to non-stress conditions following the low temperature and light treatments.

6.3.2 Material and methods

The experiment was carried out with the chlorosis-resistant variety Caldera 433, the chlorosis-sensitive variety Beersel and the single-cross hybrid SH10. Plants were grown in pots (diameter 16 cm) filled with potting compost and reared under non-stress conditions in a greenhouse until the 3rd leaf appeared. The plants were then placed in a conditioned greenhouse with an air temperature of 10 °C for 14 days (18 February – 4 March). In this greenhouse natural light was screened to create three light intensities:

- 100 % light; no screening.
- 50 % light; screened by one layer of cheese cloth.
- 20 % light; screened by three layers of cheese cloth.

Twelve plants of each genotype were used in each treatment. The pots were covered with aluminium foil to diminish warming of the soil and the shoot meristem by solar irradiation. At the end of this stage of the treatment the degree of chlorosis was estimated by visual observation. The plants were then exposed to non-stress conditions in a greenhouse for one week to study recovery from chlorosis. After that period shoot dry weight was determined.

6.3.3 Results and discussion

Visual observations during exposure to 10 °C showed that the plants became much more chlorotic at a light intensity of 100 % than 20 %. Light intensity also affected plant morphology: plants of the 20 % light-intensity group were more upright and seemed to be taller than those of 100 % light-intensity group (Figure 16). Plants of the

Figure 16. Plants of cv. Beersel after 14 days at 10 °C with 100% and 20% of the natural light intensity.

50 % light-intensity group were not intermediate; their morphology and chlorosis resembled more the 20 % light-intensity group than the 100 % light-intensity group. At 100 % light intensity, cv. Beersel was severely chlorotic and showed symptoms of leaf necrosis; SH10 was less chlorotic and cv. Caldera 433 was the most green. The differences in chlorosis between genotypes within a light intensity group were much smaller than the differences between the light treatments.

Most of the chlorosis disappeared when the plants were tranferred to non-stress conditions, but plants of cv. Beersel of the 100 % light-intensity treatment did not completely recover. Final shoot dry weight data are presented in Table 37. The differences between genotypes and treatments were not statistically significant. Shoot dry weight tended to be lower at 100 % than at 50 % light intensities. Shoot dry weight reduction as a result of high light intensity showed no clear relationship with genotypic differences in chlorosis.

The shoot dry weight data should be considered preliminary for two reasons. First, plant numbers were small. Second, no distinction can be made between direct effects of the light treatments during the 10 °C exposure and the after-effects of the induced stress.

The main conclusion is that chlorosis in plants grown in a greenhouse with an air temperature of 10 °C can be decreased considerably by lowering the light intensity.

Table 37. The effects of different light intensities during cold treatment (different degrees of chlorosis) on shoot dry weight in seedlings of cv. Caldera 433, SH10 and cv. Beersel. Temperature treatment from sowing to harvest: 14 days greenhouse, mean temperature 16 °C; 14 days greenhouse, 10 °C with light intensity treatments of 100 %, 50 % and 20 %; 7 days greenhouse, mean temperature 18 °C.

Light intensity during 10 °C exposure	Final shoot dry weight ($\bar{x} \pm s_{\bar{x}}$, mg/plant)			
	Caldera 433	SH10	Beersel	mean
100 %	108 ± 6.4	122 ± 5.2	116 ± 4.7	115
50 %	128 ± 4.2	125 ± 7.5	122 ± 6.0	125
20 %	120 ± 4.0	122 ± 5.1	112 ± 6.0	118

6.4 The effects and after-effects of light intensity during cold treatment on chlorosis and shoot growth

6.4.1 Introduction

The preceding experiment, described in Section 6.3, showed that increase of light intensity increased the degree of chlorosis in seedlings grown at the same low temperature regime. The experiment described in this section was done to investigate the effects of high and low light intensity during a 14-day exposure to low temperature on leaf extension and shoot dry matter accumulation. Then the seedlings were exposed to non-stress field conditions to study after-effects of the treatments on shoot dry matter accumulation.

6.4.2 Material and methods

The experiment was done with the varieties Caldera 433 (chlorosis resistant) and Beersel (chlorosis sensitive). The experimental design was largely similar to that described in Section 6.3 (see Table 38). Four seeds were sown in large pots (diameter 18 cm), the plants were thinned to two plants in each pot before the start of the cold treatment. At the start of the experiment there were 100 pots (200 plants) of each variety. The plant were reared under non-stress conditions for 13 days: from 7 – 16 May in a greenhouse and from 16 – 20 May outdoors. Of each variety one group of 20 pots (40 plants) was used to determine initial shoot dry weight (Harvest I). The remaining pots were placed in a greenhouse with an air temperature of 10 °C for 16 days. During this cold treatment 40 pots were exposed to 100 % and 40 pots to 20 % of the natural light intensity. The elongation rate of the 4th leaf was determined by measuring the distance between leaf tip and soil on the 2nd, 9th and 16th day of the treatment. After the cold treatment, one plant of each pot was used to determine

shoot dry weight (Harvest II). The remaining plants were exposed to field conditions for 11 days, from 5 – 16 June 1975; the air temperature for that period is given in Figure 1. Final shoot dry weight (Harvest III) was determined on 16 June. RSGR during and after the cold treatment was calculated from the mean shoot dry weight at the subsequent harvest times. Chlorosis was assessed by visual observation. During the cold treatment, air and soil temperatures were measured with thermocouples; plant temperature was measured by a thermocouple in the whirl of the plants.

6.4.3 Results and discussion

At the start of the low temperature treatment, plants were larger than in the preceding experiment (Section 6.3). In nearly all plants the tip of the 4th leaf was visible.

The cold treatment was in a period of bright weather. The air temperature in the greenhouse was around 10 °C for most of the time but on sunny days midday values of 13 – 14 °C were measured. Plant and soil temperature were also affected by solar irradiation. During the day average minimum temperatures for plant and soil were around 9 °C. The average daily maxima for plant temperature were 20 °C at 100 % light intensity and 15 °C at 20 % light intensity; soil temperatures were 14 °C and 12 °C, respectively. Plant and soil temperatures during the night were 9 – 10 °C.

During the cold treatment plants became chlorotic in the 100 % light intensity group; cv. Beersel was more chlorotic than cv. Caldera 433. In both varieties very little chlorosis was observed at 20 % light intensity, although plant temperatures were often lower than at 100 % light intensity. After transfer to the field the chlorotic plants of cv. Caldera 433 of the 100 % light intensity group recovered within four days. Plants of the 20 % light intensity group of cv. Caldera 433 became, however, more chlorotic than the 100 % light intensity group. In cv. Beersel a high degree of chlorosis was found under field conditions in both light intensity groups. Figure 1 (5 – 9 June) shows that the temperature in the field was rather high and no further development of chlorosis was expected, therefore. It appeared, however, that in both varieties the low light pretreatment had increased the sensitivity to chlorosis considerably. This sensitivity is atrributed the effects of the high light intensity in the field, as the temperature was above the stress range. A similar effect, referred to as photobleaching, is also found in other plant species upon transfer from shade to full light (Björkman, 1981).

The elongation rates of the 4th leaf during the cold treatment are given in Table 38. Leaf elongation rates were significantly lower at 100 % than at 20 % light intensities in both varieties, although the temperature of the soil and the shoot meristem was incidentally higher at 100 % light intensity. Leaf elongation rates significantly increased from the first to the second week at 100 % and 20 % light intensities in cv. Caldera 433 and at 20 % light intensity in cv. Beersel. Such an increase is normal under non-stress conditions (Figure 15). In the 100 % light intensity group of cv. Beersel a significant decrease was found, however, which may be considered a deleterious effect of the

Table 38. The effects of light intensity during a cold treatment on shoot dry matter accumulation and elongation rate of the 4th leaf in seedlings of cv. Caldera 433 and cv. Beersel. Shoot dry weight data are means of 40 plants at harvest times I, II and III. RSGR is an approximation of relative growth rate of the shoot. Leaf elongation data are from 80 plants ($\bar{x} \pm s_{\bar{x}}$) derived from measurements at the 2nd, 9th and 16th day during the 10 °C treatment. Environmental conditions were: from sowing to Harvest I, 13 days non-stress conditions; from Harvest I to Harvest II, 16 days greenhouse with an air temperature of 10 °C and 100 % or 20 % of natural light intensity; from Harvest II to Harvest III, 11 days in the field.

Cultivar	Light	Shoot dry weight (mg/plant)			RSGR ($g\,g^{-1}\,d^{-1}$)		Leaf elongation rate (mm/week) at 10 °C	
	I – II	I	II	III	I – II	II – III	1st week	2nd week
Caldera 433	100 %	113	293	2116	0.060	0.180	52.6 ± 0.87	55.4 ± 0.88
	20 %	113	345	2301	0.070	0.173	55.7 ± 0.95	60.6 ± 0.77
Beersel	100 %	123	299	1730	0.056	0.160	50.2 ± 0.93	47.6 ± 0.79
	20 %	123	307	1825	0.057	0.162	54.5 ± 0.77	57.0 ± 0.88

high light intensity. It can be concluded that a high light intensity in combination with low temperature adversely affects leaf elongation, particularly in the cold-sensitive cv. Beersel.

Shoot dry weight at the successieve harvest times and RSGR during and after the cold treatments are given in Table 38. The experimental error was rather high and no significant differences were found for shoot dry weight between varieties and light intensities within each harvest. Shoot dry matter accumulation during the cold treatment (RSGR I-II) was hardly affected by a lowering of the light intensity in cv. Beersel. The data of cv. Caldera 433, however, strongly suggest a higher RSGR at the lower light intensity. Seed reserves are usually depleted in this stage of development (Section 5.4). This implies that net photosynthesis was higher at 20 % than at 100 % light intensity. The relatively low net photosynthesis in the 100 % light group can be partly explained by the higher proportion of chlorotic leaf area in that group (Alberda, 1969). It seems likely that photosynthesis at 100 % light was mainly decreased by light-dependent chilling damage to the photosynthetic apparatus other than chlorosis (see further Section 9.3). RSGR after the cold treatment (RSGR II-III) showed little effect of the light level in cv. Beersel. In cv. Caldera 433, however, shoot dry matter accumulation tended to be higher after exposure to 100 % than 20 % light intensity, which may be associated with the higher degree of chlorosis developed in the field in the latter group.

The main objective of this experiment was to find out how high-light-induced chlorosis affected shoot dry matter accumulation in two varieties that differ in resistance to chlorosis. RSGR tended to be higher in the cold resistant cv. Caldera 433 than in the cold sensitive cv. Beersel during and after the cold treatment in both light intensity groups. RSGR in cv. Beersel was hardly affected by the light treatments. In

(Section 6.4) but the data are not fully comparable since the light levels during the post-stress period out of doors were much higher than in the present growth-chamber trial. It should be noted that total chlorophyll of the plants exposed to 16/10 °C and 55 W m^{-2} also increased upon exposure to 20/10 °C, indicating that a day temperature of 16 °C was suboptimal for chlorophyll accumulation. The chlorophyll *a/b* ratio was little affected during the post-stress treatment.

The effects and after-effects of the three cold treatments on the elongation rate of the 3rd leaf (LER) are presented in Table 40, the average data in Table 42. Leaf elongation rate at 10/16 °C was only slightly lower than at 16/10 °C at the beginning of the cold treatments. At 16/10 °C, however, it increased with time whereas at the 10/16 °C treatments it was rather constant in SH3 and it decreased in SH7 (see also Figure 15). At the end of the cold treatments, leaf elongation rate was significantly higher at 16/10 °C than at 10/16 °C; in the latter treatment lowering of the light intensity had little effect. At 20/10 °C, leaf elongation rate increased considerably after all cold treatments; this increase continued during the 20/10 °C exposure. However; clear differences can be seen between the pretreatments. It appears that 10/16 °C had a negative after-effect, particularly in combination with high light intensity. SH7 had a higher leaf elongation rate than SH3 during and after all cold treatments. Genotypic differences in response to the treatments were small or absent (Table 42).

Data on shoot dry matter accumulation are presented in Table 41. Initial shoot dry weight was much higher in SH7 than in SH3. Within the genotypes the 10/16 °C treatments caused similar effects. Shoot dry weight is reduced by 16 – 20 % at 10/16 °C and 55 W m^{-2} and by 28 – 30 % at 10/16 °C and 18 W m^{-2} in comparison with 16/10 °C (see also RSGR I-II). It seems likely that shoot growth at 10/16 °C and 18 W m^{-2} suffered from shortage of carbohydrates due to the low light level. Upon transfer to a temperature regime of 20/10 °C RSGR increased considerably in all treatments. After the 16/10 °C treatment RSGR was much higher in SH7 than in SH3. To assess after-effects of the 10/16 °C treatments, data of RSGR II-III of these treatments are compared with those of 16/10 °C (see also Table 42). The lowest RSGR was found in both genotypes after treatment at 10/16 °C and 55 W m^{-2}, whereas RSGR after 10/16 °C and 18 W m^{-2} was clearly higher than that after 10/16 °C and 55 W m^{-2} and in SH3 even higher than that after 16/10 °C. It can be concluded that recovery from damage by low day temperature is promoted by a low light intensity during cold treatment.

The main data of the experiment are summarized in Table 42, to consider relationships between RSGR, leaf elongation rate and chlorophyll content during the cold treatments and at the exposure to 20/10 °C. First, mean data of the two genotypes will be discussed, to evaluate overall effects of the treatments. At 10/16 °C and 55 W m^{-2}, RSGR and leaf elongation rate were lower than at 16/10 °C and 55 W m^{-2}. This difference is attributed to lower photosynthesis at 10/16 °C, which is probably caused by the lower chlorophyll content. At 10/16 °C, lowering of the light intensity from 55 to 18 W m^{-2} resulted in a clear decrease of RSGR and a slight decrease of leaf elongation rate. Here again the lower RSGR is attributed to a lower photosynthesis, but now

Table 40. The effects and after-effects of three climate treatments on the elongation rate of the 3rd leaf (LER) in SH3 and SH7. Data are $\bar{x} \pm s_{\bar{x}}$ from the same group of 20 plants within each treatment.

Geno-type	Treat-ment	Treatment conditions		LER (mm d^{-1}) 9 days A, B, C			LER (mm d^{-1}) 5 days 20/10 °C	
		temperature (°C)	light intensity (W m^{-2})	Day 3 – 4	Day 5 – 6	Day 7 – 9	Day 2 – 3	Day 4 – 5
SH3	A	16/10	55	8.6 ± 0.14	9.3 ± 0.23	9.8 ± 0.20	23.5 ± 0.43	30.4 ± 0.57
SH3	B	10/16	55	7.7 ± 0.23	7.5 ± 0.17	7.2 ± 0.17	16.7 ± 0.37	24.2 ± 0.46
SH3	C	10/16	18	7.1 ± 0.13	7.0 ± 0.15	7.4 ± 0.15	18.6 ± 0.32	27.7 ± 0.60
SH7	A	16/10	55	10.7 ± 0.28	11.1 ± 0.26	11.6 ± 0.22	27.8 ± 0.64	34.5 ± 0.38
SH7	B	10/16	55	10.4 ± 0.23	9.4 ± 0.24	8.7 ± 0.22	18.9 ± 0.35	28.9 ± 0.37
SH7	C	10/16	18	9.2 ± 0.26	8.5 ± 0.25	8.2 ± 0.22	21.8 ± 0.47	31.2 ± 0.47

Table 41. The effects and after-effects of three climate treatments on shoot dry matter accumulation in SH3 and SH7. Shoot dry weight data ($\bar{x} \pm s_{\bar{x}}$) are from Harvest I, II and III (see Subsection 6.5.2). RSGR I – II is during the climatic treatments, RSGR II – III after those treatments at 20/10 °C.

Geno-type	Treat-ment	Treatment conditions		Shoot dry weight (mg/plant)			RSGR (g g^{-1} d^{-1})	
		temperature (°C)	light intensity (W m^{-2})	I	II	III	I – II	II – III
SH3	A	16/10	55	42.1 ± 1.27	100.8 ± 3.30	215.2 ± 5.66	0.097	0.152
SH3	B	10/16	55		84.9 ± 1.39	162.7 ± 4.73	0.078	0.130
SH3	C	10/16	18		73.0 ± 1.83	164.0 ± 3.80	0.061	0.162
SH7	A	16/10	55	57.2 ± 1.73	144.2 ± 3.52	330.1 ± 8.29	0.094	0.182
SH7	B	10/16	55		106.7 ± 2.68	226.4 ± 4.76	0.069	0.150
SH7	C	10/16	18		93.7 ± 2.60	219.3 ± 5.90	0.055	0.170

Table 43. The effects of duration of chilling at 4 °C and of the stage of development on survival of seedlings. The data are mean survival percentages of four varieties (10 seedlings per variety).

Duration of chilling (days)	Stage of development		
	2nd leaf	4th leaf	5th leaf
3	100	100	100
6	100	52	0
9	45	0	0

was just above the shoot meristem during chilling (see Figure 17). Six days of chilling killed all seedlings in the 5th-leaf stage. In seedlings in the 4th-leaf stage, most of the leaf tissue was killed, but ultimately half of the seedlings recovered by developing new leaves. Leaf damage also occurred in the 2nd-leaf stage but those seedlings soon recovered, and all survived. Nine days of chilling caused even greater damage: only plants of the 2nd-leaf stage recovered. It can be concluded that chilling damage increased with duration of the cold treatment and with the stage of seedling development. It is likely that recovery is promoted by the amount of seed reserves.

There were slight differences between the varieties in survival and leaf damage; those differences did not correlate, however, with differences in chlorosis resistance.

An additional experiment was carried out with the 10 single-cross hybrids. Seedlings in the 4th-leaf stage were exposed to 4 °C in the dark for 6 days. The results were similar to those of the first trial. No relationship was found between the amount of damage and the genotypic variation in resistance to chlorosis or chilling before emergence.

6.6.2 The effects of continuous and diurnal chilling treatments

The main objective of this experiment was to investigate the effects of cold nights (4 °C) in combination with low and intermediate day temperature. In addition, effects of continuous chilling treatments, 4 °C in the dark, for 3 and 6 days, were tested to compare the effects of diurnal and continuous chilling. Seedlings of the single-cross hybrids SH2, SH3 and SH10, and seedlings of cv. Fronica were reared in a greenhouse kept at a moderate temperature. When the 3rd leaf appeared (in cv. Fronica often the 4th leaf had already appeared) cold treatments were started; in one treatment (Table 44, Treatment C) seedlings of the 4th-5th leaf stage were tested. In each treatment, eight seedlings of each genotype were tested. The continuous chilling treatments (Treatments A-C) were done in a dark room and the diurnal chilling treatments (Treatments D-G) in a growth cabinet where the light intensity was 55 W m^{-2} and the day length 12 h. The total period of 4 °C in the diurnal treatments of 7 days

Table 44. The effects of various cold treatments on seedlings of SH2, SH3, SH10 and cv. Fronica. The seedlings were reared in a greenhouse, exposed to cold treatments in the 3rd-leaf stage (except in C), then exposed to greenhouse conditions. Shoot dry weight was estimated when the 5th leaf appeared; relative shoot dry weight was based on shoot dry weight of a control group not exposed to a cold treatment.

Treatment	Damage	Range of resistance	Range relative shoot dry weight
A 4 °C, 3 days	leaf necrosis	no differences	SH2 > SH3 > Fronica > SH10
B 4 °C, 6 days	severe leaf necrosis	SH2 > SH10 > Fronica > SH3	SH2 > SH10 > Fronica > SH3
C 4 °C, 3 days (4th – 5th leaf)	leaf necrosis cross-bands	SH10 > SH2 > Fronica > SH3	SH10 > Fronica > SH2 > SH3
D 10/4 °C, 7 days	some cross-bands	no differences	SH2 > Fronica > SH10 > SH3
E 10/4 °C, 14 days	chlorosis cross-bands	SH2 > SH3 > Fronica > SH10 SH10 > SH2 > SH3 > Fronica	SH2 > SH3 > Fronica > SH10
F 16/4 °C, 7 days	no damage	no differences	Fronica > SH10 > SH2 = SH3
G 16/4 °C, 14 days	no damage	no differences	Fronica ≥ SH2 ≥ SH3 ≥ SH10

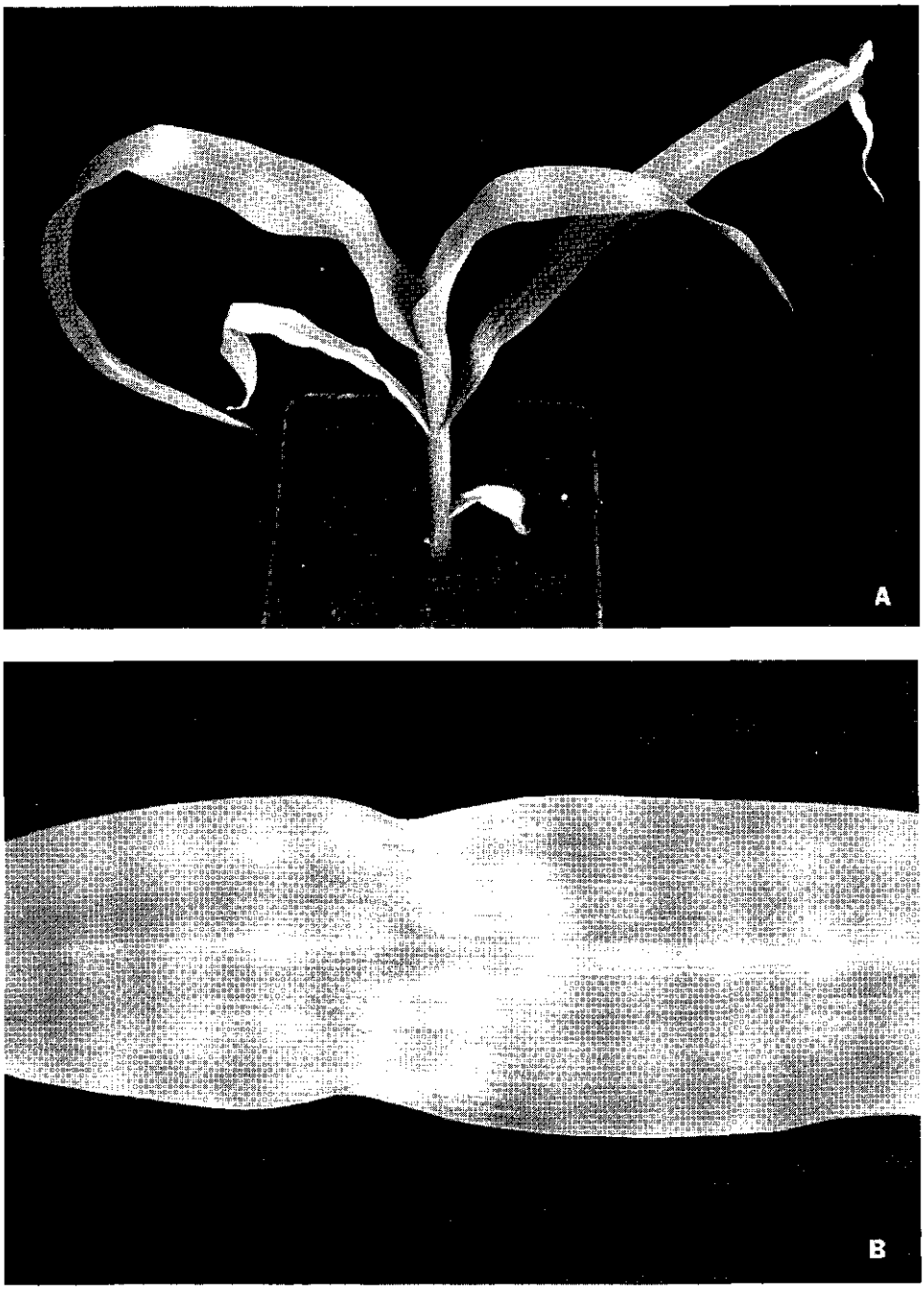

Figure 17. Plant (A) and part of 4th leaf (B) of cv. Fronica exposed to 4 °C for 3 days when the 5th leaf appeared (Treatment C), then grown in a greenhouse for 5 days. Note the chlorotic cross bands in the leaf blades of the 3rd, 4th and 5th leaf, and the dead leaf parts of the 1st, 2nd and 3rd leaf.

(Treatments D and F) was about 3 days – as in the continuous Treatment A and in the diurnal treatments of 14 days (Treatments E and G) it was about 6 days – as in the continuous Treatment B. After the cold treatments the seedlings were returned to the greenhouse to estimate damage and subsequent recovery, if any. Shoot dry weight was determined at about the 5th-leaf stage. Relative shoot dry weight was estimated on the basis of a control group that had remained in the greenhouse until the 5th-leaf stage.

The results are summarized in Table 44. First, we will consider the effects of the continuous 3 to 6-day-chilling treatments (Treatments A, B and C). All plants, except 3 plants of SH3, survived 6 days of chilling (Treatment B). Leaf necrosis after the treatments was similar to that observed in the preceding experiment (Subsection 6.6.1). Characteristic narrow cross-bands were only observed in seedlings chilled in the 4th-5th leaf stage (Treatment C, Figure 17). Green veins were often seen in the cross-bands, which indicates that the bundle sheath cells were more chilling-resistant than the mesophyll cells. Necrotic tissue was not restricted to the cross-bands: some mature leaf tissue was also destroyed. After 6 days of chilling (Treatment B) a large proportion of the leaves died. Less damage was found after 3 days of chilling (Treatment A). Figure 18 shows that there is some resemblance between the leaf damage after 3 days of chilling in the 3rd-leaf stage (Treatment A) and the cross-bands and in

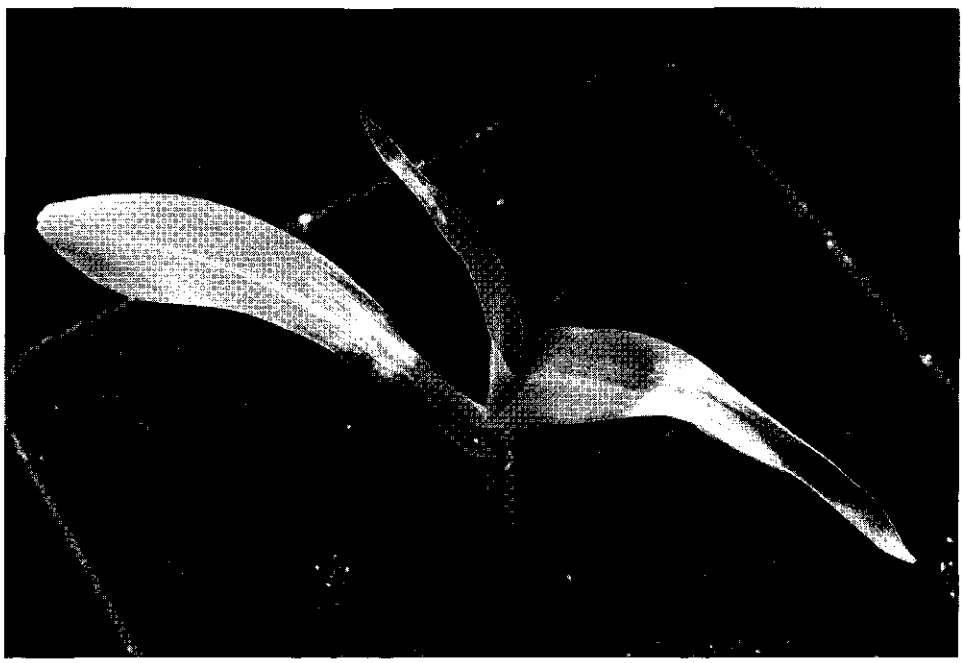

Figure 18. Plant of SH3 exposed to 4 °C for 3 days when the 3rd leaf appeared (Treatment A), then grown in a greenhouse for 4 days.

the 4th-5th leaf stage (Treatment C). The damage was found in the younger leaf parts but the necrotic area was larger and more irregular than the narrow cross-bands of Treatment C. Different degrees of damage were observed in plants of one genotype within a treatment. An estimate of total chilling resistance (i.e. leaf survival and plant recovery) showed differences between genotypes after 6 days of chilling (Treatment B) and after 3 days of chilling in seedlings of the 4th-5th leaf stage (Treatment C), but not in seedlings in the 3rd-leaf stage (Treatment B). The range of this chilling resistance was largely similar to the range of relative shoot dry weight of the genotypes.

Second, we will consider the effects of cold nights (Table 44, Treatments D – G). A sequence of nights with a temperature of 4 °C caused no visible damage when the day temperature was 16 °C (Treatments F and G). At a day temperature of 10 °C some small cross-bands developed after a 7-day treatment (Treatment D), and large cross-bands after a 14-day treatment (Treatment E). Even in the 14-day treatment damage was less than after 3-6 days exposure to 4 °C (Treatments A – C). In Treatment E, the chlorotic leaf area that developed during the cold treatment did not recover completely during subsequent growth under non-stress conditions (Figure 19). Once more green veins were observed in these cross-bands. Genotypic differences in damage were only observed after 14 days of exposure to 10/4 °C (Treatment E). The range of chlorosis

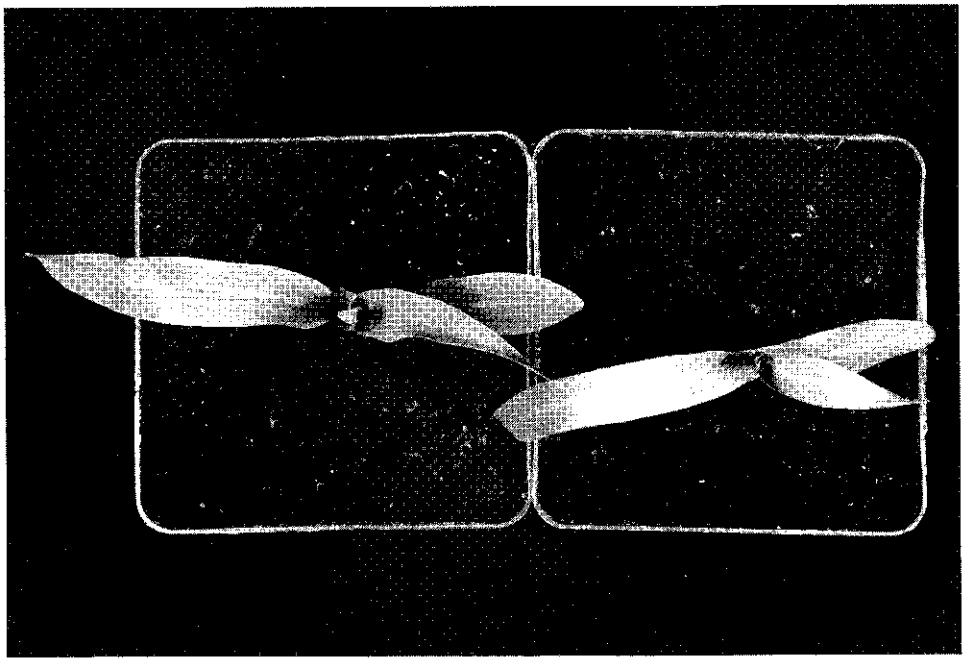

Figure 19. Plants of SH2 (left) and SH10 (right) exposed to 10/4 °C for 14 days (Treatment E), then grown in a greenhouse for 4 days. The chlorotic part of the 3rd leaf developed during the cold treatment.

during cold treatment agreed with the range of relative shoot dry weight, but not with the range of cross-bands.

6.6.3 Conclusions

Continuous chilling treatments (4 °C, for 3 – 9 days) resulted in different types of damage. Nine days of chilling kills the plants completely. After 3 – 6 days of chilling the leaves die. A particular type of injury is narrow, necrotic cross-bands. The damaged leaf tissue does not recover, but plants survive by developing new leaves. The injury increases with duration of the chilling treatment and the stage of seedling development. Mature leaf tissue may die (Figure 17), but immature tissue is specially sensitive (Figure 18). Narrow cross-bands develop in tissue that is just above or in the shoot meristem during chilling.

A sequence of 7 – 14 nights of 4 °C does not cause visible damage when the day temperature is 16 °C. With day temperatures of 10 °C leaves did, however, become chlorotic during a 14-day treatment. This chlorosis does not completely recover after return to non-stress conditions, so that wide chlorotic cross-bands remain (Figure 19).

Genetic differences were small and not very consistent because of differences between plants within one group. No clear relationships with sensitivity to chlorosis or chilling before emergence were found. The chilling treatments used in these experiments are, therefore, of no use for screening for cold tolerance for breeding purposes.

6.7 Discussion

6.7.1 Chlorosis

The physiological backgrounds of chlorosis have been discussed earlier (Miedema, 1982; see also McWilliam, 1983). Maize plants become chlorotic when they are grown at temperatures that just allow some leaf extension but are too low for normal chlorophyll accumulation. Chlorosis is the result of photo-oxidation of chlorophyll in developing leaf tissue; this is promoted by high light intensity.

Chlorosis occurs when the temperature during the day is between 7 and 16 °C. At 7 – 10 °C, leaf extension is very slow as is the development of chlorotic leaf area (Section 5.5). At 16 °C and a light intensity of 55 W m^{-2} no chlorosis was observed, although chlorophyll concentration was lower than at 20 °C (Section 6.5). However McWilliam & Naylor (1967) showed that chlorophyll accumulation at 16 °C decreased to zero when the light intensity increased to 175 W m^{-2}. Those data show that chlorosis is the result of interaction between temperature and light intensity. At higher temperatures a higher light intensity is required to inhibit chlorophyll accumulation.

Genotypic differences in chlorosis resistance were observed under intermediate stress conditions, although the effects of genotype were small in comparison with the influence of light intensity (Sections 6.3 and 6.4). An effective screening can be carried out at 14/8 °C (day/night) in a growth chamber (data not presented), or in a

7 The effects of low temperature on seedling growth of various landraces and CIMMYT gene-pool populations

7.1 Introduction

The preceding chapters describe effects of low temperature on ten single-cross hybrids of Northern flint and Corn Belt dent origin and on some varieties based on the same groups of materials. Those flint and dent accessions are, however, a very small proportion of the maize germplasm. This chapter describes experiments with material of a much broader genetic basis. The material consisted of various landraces from Mexico, USA and Europa, and gene-pool populations developed for tropical highlands and tropical lowlands by CIMMYT (International Maize and Wheat Improvement Center).

A first experiment (Field Experiment) investigated the effects of low temperature on seedling growth in 19 accessions by comparing the effects of early and late sowing. A brief account of this experiment has been published earlier by Miedema (1979). A second experiment (Chilling Experiment) investigated resistance to chilling before emergence in 11 of the above accessions. This experiment was done to find out whether the positive correlation between seedling growth at low temperature and chilling resistance found in the 10 single-cross hybrids (Chapter 4) also applies to other materials. The 11 accessions of the chilling experiment were also tested for rate of germination and emergence at 12 °C. The data of this test (already presented in Section 3.4) and the data of the experiments described in this chapter will be considered in a discussion on relationships between cold tolerance traits.

7.2 Material and methods

Plant material. Nineteen accessions and two single-cross hybrids were used in the Field Experiment (Table 46), and 11 accessions and three single-cross hybrids in the Chilling Experiment (Table 48). The accessions consisted of landraces from various regions and CIMMYT gene-pool populations (Anonymous, 1974) with wide differences in climatic adaptations. The tropical highland material is particularly interesting because of its high potential production under cool maritime conditions (Ahloowalia, 1973). All seed material was multiplied in the greenhouse.

Field experiment. Seedling growth at early and late sowing. Sowing dates were 28 April (Trial I) and 22 July (Trial II). Of each accession, 24 kernels (6 replicates of 4 seeds) were sown in pots (diameter 16 cm) containing potting compost. The pots were

put outdoors and watered when necessary. Air temperature at plant level was measured with a shaded thermograph.

Dates of emergence were recorded for individual plants. Shoot length after emergence was measured on 21 May in Trial I and on 2 August in Trial II. In Trial I, plant size and resistance to chlorosis were assessed by visual observation on 25 May (3rd- to 4th-leaf stage). Finally, shoot dry weight was determined when, for most of the plants, the 6th leaf had become visible; harvest dates were 9 June for Trial I and 17 August for Trial II. Aberrant plants were omitted from the calculation of average data of the various characteristics; final plant numbers ranged from 19 – 24.

Ratios of the data of the early and the late sowing (i.e. data Trial I/data Trial II) were calculated for time to emergence, shoot length after emergence and shoot dry weight. These ratios are an approximation of the response to low temperature independent of genotypic differences in vigour.

Chilling experiment. Chilling resistance during early seedling growth. Chilling trials were carried out with germinated seedlings according to the technique described in Section 4.2. The following treatments were tested:
Treatment A. 3 days 20 °C, 6 days 4.0 °C, 3 days 20 °C.
Treatment B. 3 days 20 °C, 6 days 2.5 °C, 3 days 20 °C.
Treatment C. 4 days 20 °C, 6 days 2.0 °C, 3 days 20 °C.

In a first test, Treatments A and B were applied; 4 replicates of 5 seeds of each genotype were used. It turned out that these chilling treatments were too mild, and that some accessions had not completely germinated at the onset of chilling. Therefore a definite trial was done with Treatment C being 4 days of 20 °C pre-treatment instead of 3 days and a chilling temperature of 2 °C; here 8 replicates of 5 seeds were used. After the treatments, occurrences of root and shoot necrosis were recorded. The degree of chilling resistance was estimated for individual plants on a 1 – 9 scale: 1 = most sensitive, 9 = most resistant.

Correlation coefficients between various characteristics of 19 accessions of the field experiment, and between cold tolerance traits of 11 accessions in the field experiment, the chilling experiment and te germination experiment of Section 3.4 were calculated.

7.3 Results and discussion

7.3.1 Seedling growth at early and late sowing (Field experiment)

Mean air temperatures and duration of the growth periods of Trials I and II are presented in Table 45. The average temperature during Trial I was rather low, but normal for Dutch conditions. In Trial II the temperature was above stress level during the whole period.

The results are presented in Table 46. The average values of the 21 accessions show the overall effects of the sowing dates, the coefficient of variation is a measure of differences between the accessions or genotypes. The correlation coefficients (Table 47)

Tabel 45. Mean air temperature during the growth periods.

Trial	Sowing until emergence		Emergence until harvest	
	period	temperature (°C)	period	temperature (°C)
I	28 April – 14 May	10.2	15 May – 9 June	13.3
II	23 July – 30 July	15.3	31 July – 17 August	16.8

between data of Trials I and II for the same characteristics show the proportion of genotype effects independent of temperature or other environmental factors associated with sowing time.

Time from sowing to emergence was on average about 17 days in Trial I and 9 days in Trial II. The coefficient of variation of time to emergence was low in Trial I, Trial II and, in particular, for the ratio Trial I/Trial II. The correlation coefficient between time to emergence in Trial I and Trial II was rather high ($r = 0.833^{**}$), which implies that about 70 % of the variation was associated with a common, genetically-determined component not affected bij sowing date or temperature. In Trial I, rapidly emerging accessions were No 4 and CIMMYT Highland materials (No.s 12, 13 and 15); slowly emerging accessions were No 3, No 11 and CIMMYT Lowland materials (No.s 18 and 19). Earlier investigations showed that such differences are accociated with mesocotyl length (Section 3.4); accessions adapted to tropical lowland such as No.s 3, 18 and 19 had very short mesocotyls when grown at 12 °C. In field trials, particularly at early sowing, such accessions had a deep shoot meristem, whereas materials adaptated to tropical highland (No.s 2, 12 and 13) has a shallow shoot meristem.

In Trial I, some chlorosis was observed but the proportion of chlorotic leaf area was relatively small. Temperatures were too high for severe chlorosis so conclusions based on the estimates should be considered preliminary. The high coefficient of variation for resistance to chlorosis is not realistic because a 1 – 9 scale was used. The same applies to the coefficient of variation of the estimate of plant size at 25 May.

Shoot length measurements were carried out about 7 days after emergence in Trial I and 4 days after emergence in Trial II. The differences between mean shoot length in Trials I and II are due to differences in stage of plant development. Shoot length is associated with rate of emergence and initial leaf extension. Its coefficient of variation was high in Trials I and II and for the ratio Trial I/Trial II. The correlation coefficient between Trial I and II was 0.777**, which implies that about 60 % of the variation was associated with a common, genetically-determined component independent of sowing date.

Shoot dry weight was determined 25 days after emergence in Trial I and 17 days after emergence in Trial II. Average shoot dry weight was 540 mg in Trial I and 880 mg in Trial II. The stage of plant development was similar, in both trials about 50 % of

Table 46. Field experiment. Average data of time to emergence, shoot dry weight and other characteristics of various landraces, CIMMYT gene-pool populations, and two single-cross hybrids, after early (Trial I) and late (Trial II) sowing. Trial I: Sowing 28 April, harvest 9 June; Trial II: sowing 22 July, harvest 17 August. Shoot length was determined on 21 May and 2 August for Trial I and Trial II, respectively.

Material[a] (and its origin)	Kernel weight (mg)	Time to emergence (days)			Resistance to chlorosis[b]	Plant size[b] 25 May	Shoot length (mm)			Shoot dry weight (mg)		
		I	II	I/II			I	II	I/II	I	II	I/II
1. Conico, Queretaro 44 (Mexico)	320	16.1	8.3	1.94	5.2	6.8	61	52	1.17	544	960	0.57
2. Conico, Zacatecas 34 (Mexico)	398	15.7	8.0	1.96	5.3	6.5	57	49	1.16	501	827	0.61
3. Chapalote-Reventador, Sinaloa 2 (Mexico)	248	18.6	9.0	2.07	3.7	4.2	41	43	0.95	259	618	0.42
4. Chalqueño, Mexico Grupo 17 (Mexico)	362	15.0	8.5	1.76	6.5	5.7	57	42	1.36	569	784	0.73
5. Tuxpeño, Queretaro Grupo 17 (Mexico)	282	15.3	8.4	1.82	6.0	5.3	52	45	1.16	444	775	0.57
6. Baileys (USA)	328	18.0	9.6	1.88	4.8	6.2	43	31	1.39	418	794	0.53
7. Vulgar de Vitago (Portugal)	334	17.1	9.0	1.90	7.3	5.8	49	38	1.29	511	945	0.54
8. Branco de Pias (Portugal)	397	17.9	9.4	1.90	7.5	7.2	45	31	1.45	628	1057	0.59
9. Rheintaler (Switzerland)	350	16.8	9.7	1.73	8.7	7.7	50	28	1.79	681	1030	0.66
10. Baanbreker (Netherlands)	311	18.1	9.8	1.85	7.7	5.0	42	22	1.91	570	791	0.72
11. Mestnaja scorospelaja (Russia)	210	19.2	9.8	1.96	4.7	2.8	34	22	1.54	348	714	0.49
12. CIMMYT HEYF pool 4	316	14.3	8.0	1.79	6.2	7.0	64	46	1.39	806	886	0.91
13. CIMMYT HEYD pool 5	312	14.6	8.5	1.72	6.0	6.7	63	40	1.58	619	688	0.90
14. CIMMYT HIYF pool 9	372	15.4	8.5	1.81	6.7	7.8	66	52	1.27	675	1088	0.62
15. CIMMYT HIYD pool 10	379	14.7	8.4	1.75	6.5	7.0	69	51	1.35	658	962	0.68
16. CIMMYT LEYF pool 17	278	18.1	9.2	1.97	2.2	5.8	46	41	1.12	412	874	0.47
17. CIMMYT LEYD pool 18	348	17.5	8.8	1.99	3.0	7.3	53	50	1.06	465	911	0.51
18. CIMMYT LIYF pool 21	380	18.8	9.3	2.02	4.8	6.7	46	34	1.35	528	989	0.53
19. CIMMYT LIYD pool 22	364	18.5	9.5	1.95	4.2	6.7	44	34	1.29	509	940	0.54
20. SH 2 (Spain, Netherlands)	209	14.9	8.2	1.82	8.5	5.8	50	42	1.19	597	883	0.68
21. SH10 (USA)	306	16.3	8.3	1.96	7.3	7.0	54	46	1.17	608	959	0.63
Average	324	16.7	8.9	1.88	5.8	6.2	51.7	40.0	1.33	540	880	0.61
Coefficient of variation (%)	16.9	9.5	6.9	5.4	29.7	19.2	17.9	23.3	17.4	23.1	14.1	20.6

[a] Abbreviations: H, highland; L, lowland; E, early; I, intermediate; Y, yellow; F, flint; D, dent.
[b] Estimates on a 1 – 9 scale: 1 is most yellow and smallest in size; 9 is most green and largest in size.

Table 47. Correlation coefficients between characteristics of 19 accessions of maize (SH2 and SH10 are omitted). Data from Table 46.
* = $P \leqslant 0.05$ ** = $P \leqslant 0.01$.

	1	2	3	4	5	6	7	8	9	10	11
1. Kernel weight											
2. Time to emergence I	−0.289										
3. Time to emergence II	−0.232	0.833**									
4. Time to emergence I/II	−0.220	0.738**	0.244								
5. Resistance to chlorosis	0.350	−0.387	0.019	−0.698**							
6. Plant size I, 25 May	0.796**	−0.435	−0.322	−0.357	0.257						
7. Shoot length I	0.450	−0.911**	−0.832**	−0.583**	0.254	0.635**					
8. Shoot length II	0.264	−0.639**	−0.885**	−0.038	−0.242	0.426	0.777**				
9. Shoot length I/II	0.044	0.015	0.484*	−0.559*	0.682**	0.005	−0.130	−0.707**			
10. Shoot dry weight I	0.570*	−0.640**	−0.333	−0.719**	0.654**	0.710**	0.679**	0.170	0.459*		
11. Shoot dry weight II	0.684**	−0.069	0.010	−0.132	0.333	0.749**	0.306	0.155	0.076	0.584**	
12. Shoot dry weight I/II	0.226	−0.736**	−0.414	−0.790**	0.537*	0.344	0.616**	0.089	0.519*	0.792**	−0.018

the plants were in the 6th-leaf stage. The differences in shoot dry weight are due to plant morphology: in Trial I the plants were shorter than in Trial II, which may be attributed to the lower temperature in Trial I (see also Sections 5.2 and 5.3). The coefficient of variation for shoot dry weight was highest in Trial I (23.1 %), but also high for the ratio of values Trial I/Trial II (20.6 %) and rather low in Trial II. The correlation coefficient between Trial I and Trial II was 0,584**, which implies that about 35 % of the variation was associated with a common factor independent of sowing date.

Although the temperature stress was not severe during Trial I, interesting differences in shoot dry weight were recorded. Tropical highland materials (No.s 12 – 15) and European landraces (e.g. No.s 8, 9 and 10) had a high shoot dry weight, but also the cold-sensitive SH10 was high in this experiment; the Trial I/Trial II shoot dry weight ratios were particularly high for the CIMMYT gene pools HEYF and HEYD. Tropical lowland materials (No.s 3 and 16 – 19) had a low shoot dry weight in Trial I and a low Trial I/Trial II ratio also. The tropical highland and lowland materials not only differed in shoot weight but also in shoot morphology. As mentioned above, the shoot meristems were deep for the lowland materials and shallow for the highland materials, which resulted in a poor standability in the latter seedlings.

Figure 20. Plants of the early sowing of CIMMYT gene-pool populations for tropical highland HEYF (E) and HEYD (G), and tropical lowland LEYF (F) and LEYD (H), one day before harvest.

Seedlings of the highland materials had a more elongated appearance because their leaf sheaths were much longer than those of the lowland accessions (Figure 20). Another morphological characteristic not directly associated with cold tolerance is tillering. Tillering was observed in seedlings of accessions 4, 5, 12 and 15; the numbers of tillering plants were higher for early than late sowing.

A discussion on relationships between cold tolerance traits of the sowing date experiment, the next chilling test and the germination and emergence test of Section 3.4 will be presented in Subsection 7.3.3. Here relationships whithin the sowing date experiment will be discussed. Table 47 presents correlations between 12 characteristics. Relative values, i.e. Trial I/Trial II ratios, of time to emergence, shoot length after emergence and shoot dry weight are considered better indicators for cold tolerance than absolute values.

The shoot dry weight Trial I/Trial II ratio correlated with the time to emergence Trial I/Trial II ratio ($r = -0.790^{**}$), resistance to chlorosis ($r = 0.537^{*}$) and the shoot length (Trial I/Trial II ratio) ($r = 0.519^{*}$). Shoot length Trial I/Trial II ratio correlated with the time to emergence Trial I/Trial II ratio ($r = -0.559^{**}$) and resistance to chlorosis ($r = 0.682^{**}$). The time to emergence Trial I/Trial II ratio correlated with resistance to chlorosis ($r = -0.698^{**}$).

Other interesting correlations were between kernel weight and intermediate plant size in Trial I estimated on 25 May ($r = 0.796^{**}$), and shoot dry weight in Trial I ($r = 0.570^{*}$) and Trial II ($r = 0.684^{**}$). The intermediate plant size estimate in Trial I correlated with shoot dry weight in Trial I ($r = 0.710^{**}$) but also with shoot dry weight in Trial II ($r = 0.749^{**}$). However, it did not significantly correlate with cold tolerance traits such as time to emergence Trial I/Trial II ratio, chlorosis resistance, shoot length Trial I/Trial II ratio and shoot dry weight Trial I/Trial II ratio, which may indicate that this shoot size, which was estimated in the 3rd leaf stage, is associated with vigour rather than cold tolerance.

7.3.2 Chilling resistance during early seedling growth

Chilling Treatment A (4.0 °C) caused very little damage; root necrosis was only observed in accession No 2. Treatment B (2.5 °C) caused more damage and also affected accession No 13, one of the most chilling-sensitive single-cross hybrids. Many accessions were hardly affected by the 2.5 °C treatment. Some accessions not fully germinated after the 3-day pre-treatment, which may have protected them from chilling damage. Treatment C (4 days pre-treatment, 2.0 °C chilling temperature) was the most succesful since a wide range of damage was observed (Figure 21). Various degrees of root and shoot damage occurred, the roots being more sensitive than the shoots. Root injury has been described in Subsection 4.2.2. Shoot injury was located in the basal tissue of the mesocotyl. The injury ranged from slight discoloration to necrosis, the latter resulting in the death of the seedlings. The data of the chilling resistance scores and main data from the preceding trials are presented in Table 48. There was a high correlation ($r = 0.881^{**}$) between chilling resistance of the shoot

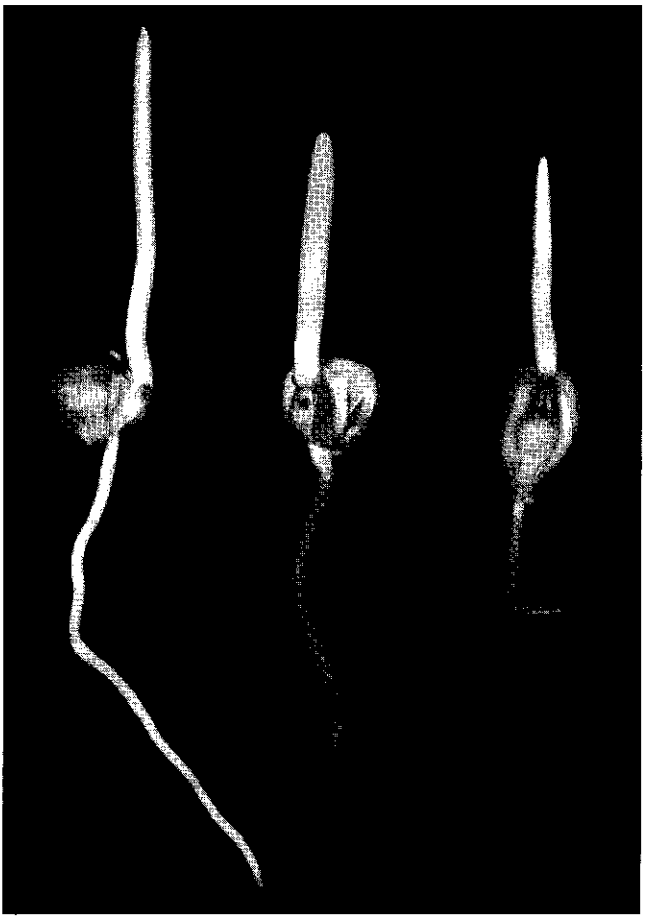

Figure 21. The effect of chilling treatment (4 days 20 °C, 6 days 2 °C, 3 days 20 °C) on seedlings of SH2 (left) with no necrosis, SH10 (middle) with root necrosis, and Chapalote-Reventador, Sinaloa 2 (right) with necrotic root and shoot basis.

and that of the root (Table 49), which may indicate a common physiological basis. Because of this high correlation we will discuss only the mean value of shoot and root resistance.

Table 48 shows that the range of chilling sensitivity of the 11 accessions was largely similar to that of the 10 single-cross hybrids, of which SH2 is the most resistant and SH10 the most sensitive. SH2 was little better than the best European landraces (No.s 9 and 10). Accession No 2, a tropical lowland accession from Mexico, was more sensitive than SH10. Of the CIMMYT gene-pools used, the tropical lowland populations had a low chilling resistance; the highland populations were better but did not exceed the European landraces. Within many populations a wide range of individual plant

Table 48. Chilling resistance, early seedling growth at 12 °C (data from Table 11) and field performance at early sowing (data from Table 46) of 11 land races and CIMMYT gene-pool populations. The data on chilling resistance are from Treatment C (4 days 20 °C, 6 days 2 °C, 3 days 20 °C); 40 seeds were used per accession. Chilling resistance is expressed on a 1 – 9 scale: 1 is most sensitive; 9 is most resistant.

	Chilling resistance ($\bar{x} \pm s_{\bar{x}}$)			Time to germination at 12 °C (days)	Time to emergence at 12 °C (days)	Field experiment		
	shoot	root	mean			days to emergence I	shoot dry weight (mg) I	shoot dry weight ratio I/II
1. Conico, Zacatecas 34 (Mexico)	5.8 ± 0.4	4.0 ± 0.3	4.9	6.3	16.7	15.7	501	0.606
2. Chapalote-Reventador, Sinaloa 2 (Mexico)	2.9 ± 0.4	1.3 ± 0.1	2.1	4.8	18.3	18.6	259	0.419
3. Chalqueño, Mexico Grupo 17 (Mexico)	7.9 ± 0.1	5.7 ± 0.3	6.8	6.7	15.9	15.0	569	0.726
4. Tuxpeño, Queretaro Grupo 17 (Mexico)	5.5 ± 0.4	3.5 ± 0.3	4.5	5.8	15.7	15.3	444	0.573
5. CIMMYT gene pool 4, HEYF	5.6 ± 0.4	5.0 ± 0.3	5.3	6.5	15.6	14.3	806	0.910
6. CIMMYT gene pool 5, HEYD	7.1 ± 0.2	5.0 ± 0.3	6.0	6.5	16.3	14.6	619	0.900
7. CIMMYT gene pool 21, LIYF	5.7 ± 0.2	2.1 ± 0.2	3.9	6.4	19.5	18.8	528	0.534
8. CIMMYT gene pool 22, LIYD	5.8 ± 0.3	2.2 ± 0.2	4.0	6.7	19.7	18.5	509	0.541
9. Branco de Pias (Portugal)	8.1 ± 0.2	6.8 ± 0.3	7.4	7.7	17.7	17.9	628	0.594
10. Rheintaler (Switzerland)	8.2 ± 0.2	6.3 ± 0.3	7.2	7.8	17.3	16.8	681	0.661
11. Mestnaja scorospelaja (USSR)	7.8 ± 0.2	6.4 ± 0.3	7.1	6.9	17.6	19.2	348	0.481
12. SH2	9.0 ± 0.0	8.1 ± 0.1	8.5	–	–	14.9	597	0.676
13. SH3	5.7 ± 0.4	3.2 ± 0.3	4.4	–	–	–	–	–
14. SH10	3.5 ± 0.3	1.3 ± 0.1	2.4	–	–	16.3	608	0.634

responses was recorded. In the CIMMYT HEYF (No 5), for instance, the scores for shoot necrosis ranged from 1 to 9 and the coefficient of variation was 45 %.

7.3.3 Relationships between the various cold tolerance traits

Table 49 shows the correlation coefficients between the major cold tolerance traits of the group of 11 accessions. First, chilling resistance during early seedling growth will be considered. Chilling resistance (its mean value) correlated with resistance to chlorosis ($r = 0.748^{**}$). Chilling resistance showed a high and positive correlation with time to germination at 12 °C ($r = 0.856^{**}$). This implies that chilling resistance is associated with slow germination at low temperature. Yet, no significant correlations were found between chilling resistance and time to emergence at 12 °C or early sowing.

Second, time to germination and emergence at 12 °C (data in Section 3.4) will be considered. For time to germination at 12 °C significant correlations were found with resistance to chlorosis ($r = 0.732^{*}$) and shoot dry weight in Trial I ($r = 0.611^{*}$). These correlations were positive, which suggests that slow germination at low temperature is associated with good seedling growth after emergence. This is surprising, but it should be emphasized that such relationships depend strongly on the material. Accession No 2, for instance, had a very short germination time at 12 °C, but otherwise poor seedling growth. If this genotype is omitted from the calculations, the correlation between germination time at 12 °C and shoot dry weight of Trial I decreased from $r = 0.611^{*}$ to $r = 0.330$ NS.

Time to emergence at 12 °C correlated with time to emergence of Trial I ($r = 0.875^{**}$), the ratio Trial I/Trial II ($r = 0.702^{*}$) and shoot dry weight of Trial I/Trial II ($r = -0.650^{*}$). The correlation between time to emergence at 12 °C and time to emergence at early sowing in the field was much lower in the 10 single-cross hybrids ($r = 0.576^{*}$, Table 14) than in the 11 accessions investigated in this chapter. It seems likely that laboratory tests with diurnal fluctuations will give a better indication of time to emergence under field conditions than tests with constant temperatures (see also Section 3.3).

7.4 General discussion

The purpose of the experiments described in this chapter was to investigate genotypic variation in cold tolerance in a group of genetically diverse materials and to analyse relationships between cold tolerance traits in this group.

First, the genotypic variation and its significance for breeding will be considered. The relatively small samples of 19 accessions used in the field experiment and 11 accessions of the chilling and germination tests showed a wide range of responses to low temperature. One of the most striking results was the high shoot dry weight of seedlings from CIMMYT gene pools developed for tropical highlands, particularly from the HEYF pool (see Miedema, 1979). Good performance of tropical-highland material in temperate conditions was reported for the first time by Ahloowalia (1973). He

Table 49. Correlation coefficients between cold tolerance traits of 11 accessions listed in Table 48 (SH2, SH3 and SH10 are omitted). Data of the traits 1, 2 and 3 are from Table 48, the traits 5 and 6 from Table 16, and the traits 4, 7, 8, 9 and 10 from Table 46.
* = $P \leqslant 0.05$ ** = $P \leqslant 0.01$.

	1	2	3	4	5	6	7	8	9
1. Chilling resistance shoot									
2. Chilling resistance root	0.881**								
3. Chilling resistance mean	0.964**	0.975**							
4. Resistance to chlorosis	0.705*	0.750**	0.748**						
5. Time to germination at 12 °C	0.900**	0.775**	0.856**	0.732*					
6. Time to emergence at 12 °C	−0.217	−0.497	−0.382	−0.459	0.010				
7. Time to emergence field I	−0.124	−0.276	−0.213	−0.428	0.017	0.875**			
8. Time to emergence field I/II	−0.585	−0.613*	−0.617*	−0.754**	−0.459	0.702*	0.770**		
9. Shoot dry weight field I	0.465	0.483	0.487	0.699*	0.611*	−0.364	−0.579	−0.697*	
10. Shoot dry weight field I/II	0.320	0.415	0.381	0.473	0.264	−0.650*	−0.841**	0.806**	0.815**

tested 27 landraces from the Mexican highlands in Ireland during May-October. The races were very late-flowering, but the total dry matter production of the best populations was twice that of European hybrid varieties. Such populations are adapted to high altitudes in the tropics that have average growing season temperatures of 15 – 17 °C (Anonymous, 1974). This low temperature adaptation also applies to growth at high latitudes. Research in New Zealand (Eagles & Hardacre, 1979a, 1979b; Eagles et al., 1983; Hardcare & Eagles, 1986), England (Sheldrick, 1980) and Northern Germany (Stamp, 1984a, 1984b) showed that high-altitude sources, particularly CIMMYT gene pools for tropical highlands, and crosses with these materials, grew faster and yielded more dry matter at low temperature than local varieties. Such material may possess cold tolerance genes that differ from the genes evolved in the best adapted Northern flint sources. Introduction of such exotic material, which stems from the centre of origin of maize, will broaden the genetic base of the breeding populations. Further research is needed to elucidate the physiological backgrounds of the low temperature adaptation of these highland populations.

Second, the relationships between cold tolerance traits of the 11 exotic accessions will be compared with the relationships found for the 10 single-cross hybrids. Table 50 shows correlations between shoot dry weight at early sowing and other characteristics for both groups of materials. In the 11 exotics, shoot dry weight of Trial I correlated with kernel weight, time to emergence of Trial I and Trial I/Trial II, intermediate plant size of Trial I and shoot dry weight of Trial II. None of these characteristics showed significant correlations in the group of 10 single-cross hybrids. In both groups of materials, correlations were similar between shoot dry weight at early sowing and resistance to chlorosis and shoot dry weight ratio. Resistance to chilling (root

Table 50. Correlations between two cold tolerance traits, shoot dry weight at early sowing and shoot dry weight ratio early/late sowing, and other plant characteristics in 11 exotics (Trial I, Trial I/Trial II) and 10 single-cross hybrids (S3, S3/S6). * = $P \leqslant 0.05$ ** = $P \leqslant 0.01$.

	Shoot dry weight		Shoot dry weight ratio	
	exotics Trial I	single-cross hybrids S3	exotics Trial I/Trial II	single-cross hybrids S3/S6
1. Kernel weight	0.570*	0.158	0.226	0.186
2. Time to emergence I, S3	−0.640**	0.277	−0.736**	0.497
3. Time to emergence II, S6	−0.333	0.352	−0.414	0.676*
4. Time to emergence I/II, S3/S6	−0.719**	−0.241	−0.790**	−0.486
5. Resistance to chilling roots (2 °C)	0.483	0.823**	0.415	0.781**
6. Resistance to chlorosis	0.654**	0.626*	0.537*	0.814**
7. Intermediate plant size I, S3	0.710**	−0.340	0.344	−0.724**
8. Shoot dry weight I, S3			0.792**	0.737**
9. Shoot dry weight II, S6	0.584*	0.220	−0.018	−0.486
10. Shoot dry weight I/II, S3/S6	0.792**	0.737**		

necrosis) showed a high correlation with shoot dry weight in the 10 single-cross hybrids but not in the 11 exotics. Chilling resistance of germinated seedlings cannot, therefore, be considered a simple screening technique for cold tolerance.

The question arises, what are the reasons of the above dissimilarities? The environmental conditions of the early sowings in the field were slightly different, although low temperature was the main constraint to shoot growth for both groups of materials. Therefore, dissimilarities in the relationships between the cold tolerance traits are mainly atrributed to differences in the genetic composition between the single-cross hybrids and the exotic accessions.

8 Path-coefficient analysis of growth at low temperature

8.1 Introduction

In the previous chapters correlation coefficients were calculated between various traits (or components) that were expected to contribute to the resultant variable, shoot dry weight at low temperature. Those simple correlation coefficients are useful to assess relationships between rather simple traits, such as time to germination and time to emergence, or resistance to chilling and resistance to chlorosis. Simple correlation coefficients are less suitable, however, to assess the contribution of various components to a resultant variable when those components are interrelated. Figure 22 shows that the effect of a Component 1 on the Resultant Variable 4 may be direct, or indirect through other components No.s 2 and 3. In such cases, path-coefficient analysis may be helpful.

The theory of path-coefficient analysis has been presented by Wright (1968) and Li (1976); for examples of its application we refer to Dewey & Lu (1959) and Bhatt (1973). It is a standardized partial regression analysis that partitions the simple correlation coefficient into direct and indirect effects. The direct effects are given by the

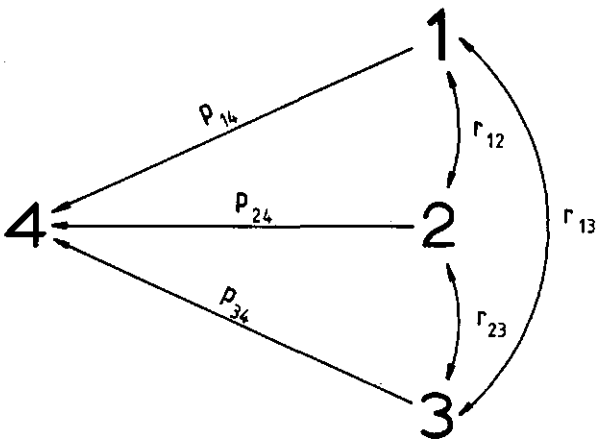

Figure 22. Path diagram with components or independent variables 1, 2 and 3, and resultant or dependent variable 4. The double-arrowed lines indicate mutual association measured by simple correlation coefficients (r), and the single-arrowed lines represent direct effects measured by path coefficients (p). (After Dewey & Lu, 1959).

so-called path coefficients (p_{14}, p_{24} and p_{34}); the indirect effects are given by the product of the simple correlation coefficient and the path coefficient (rp). The indirect effect, for instance, of Component I via Component 2 on Resultant Variable 4 is calculated by $r_{12} \times p_{24}$. The partition of the simple correlation coefficient between Component 1 and the Resultant Variable 4 is then:

$r_{14} = p_{14} + r_{12}p_{24} + r_{13}p_{34}$

The coefficient of multiple determination, R^2, gives the part of the variation of the resultant variable that is determined by its components. In the next sections, path-coefficient analysis will be presented for shoot dry weight at low temperature of the group of single-cross hybrids.

8.2 Shoot dry weight of nine single-cross hybrids at treatments of 15/10 and 20/15 °C

Path coefficient analysis was carried out for shoot dry weight in the 6th-leaf stage of nine single-cross hybrids exposed to 15/10 °C and 20/15 °C after emergence (Section 5.2). Components chosen were initial shoot elongation rate and elongation rate of the 3rd leaf (data from Table 24), and resistance to chlorosis assessed in the 1975 field experiment (data from Table 8). The results (Table 51) show that R^2 was rather high for both treatments.

The data of the 15/10 °C treatment show that the path coefficients differ from the simple correlation coefficients. This applies particularly to initial shoot elongation rate. The low and negative simple correlation between initial shoot elongation rate and shoot dry weight was the result of a positive direct effect ($p = +0.925$) and a negative and stronger indirect effect via resistance to chlorosis ($rp = -1.266$). This negative indirect effect was due to the coincidence of sensitivity to chlorosis and rapid inital shoot growth in the dent hybrids SH6 to SH10. The path coefficient suggests that initial shoot elongation rate may have a considerable positive effect on shoot dry

Table 51. Path-coefficient analysis of shoot dry weight of nine single-cross hybrids grown in growth chambers at 15/10 and 20/15 °C.

	Components	r_{simple}	p (values in italics) and rp		
			1	2	3
15/10 °C	1 Initial shoot elongation rate	− 0.276	+ *0.925*	+ 0.063	− 1.266
	2 Leaf elongation rate	+ 0.671	+ 0.156	+ *0.374*	+ 0.140
	3 Resistance to chlorosis	+ 0.615	− 0.831	+ 0.037	+ *1.409*
	$R^2 = 0.864$				
			1	2	3
20/15 °C	1 Initial shoot elongation rate	+ 0.751	+ *0.592*	+ 0.140	+ 0.019
	2 Leaf elongation rate	+ 0.737	+ 0.137	+ *0.605*	− 0.005
	3 Resistance to chlorosis	− 0.298	− 0.379	+ 0.110	− *0.029*
	$R^2 = 0.900$				

weight. The direct effect of leaf elongation rate was rather low ($p = +0.374$). Resistance to chlorosis had the highest path coefficient ($p = +1.409$), which is partly due to a negative indirect effect through initial shoot elongation rate.

For the 20/15 °C treatment, the pattern of simple correlation coefficients resembled that of the path coefficients. In contrast to the 15/10 °C treatment, no direct effect of chlorosis resistance was found. Initial shoot elongation rate and leaf elongation rate were the main components.

The data of Table 51 suggests that variation in shoot dry matter accumulation at 15/10 °C is mainly determined by initial shoot elongation rate and resistance to chlorosis. The effect of initial shoot elongation is considered a causul effect because it agrees with physiological data on seedling growth during the exponential phase. The effect of sensitivity to chlorosis is probably also causal. Although most of the plants were not visibly chlorotic, the sensitive genotypes may have suffered more from the growth crisis at depletion of seed reserves than the chlorosis-resistant hybrids (Section 5.4).

8.3. Shoot dry weight of ten single-cross hybrids and its components in the 1975 field experiment

In this section we consider shoot dry weight of the 16 April sowing (S3) of the 1975 field trial (see Chapter 2). The components chosen were kernel weight, time to emergence in S3, number of emerged plants in S1, shoot dry weight ratio ½(S1 + S2)/S3, resistance to chlorosis and shoot dry weight in S6. Table 52 shows that R^2 was rather high and that the path coefficients show a pattern of relationships slightly different from the simple correlations. Resistance to chlorosis had the strongest direct effect ($p = +1.089$), whereas kernel weight, shoot dry weight in S6 (vigour component) and pre-emergence vigour reduction (component 4) each had minor direct effects. A negligible direct effect was found for time to emergence and pre-emergence survival (Component 3). Differences between the simple correlations and the path coefficients for Components 2, 4 and 6 were mainly due to the indirect effects of these components through resistance to chlorosis (Column 5, Table 52). A physiological relationship between resistance to chlorosis and the Components 2, 4 and 6 is very unlikely, therefore the indirect effects may be attributed to coincidence of characteristics. The main conclusion from the data is that resistance to chlorosis had the strongest effect on shoot dry weight.

8.4 Compund path analysis of shoot growth of nine single-cross hybrids

In the previous section, shoot dry weight of S3 in the 1975 field experiment was analysed for components that influenced seedling growth in different stages of development. Investigation of relative shoot growth rate (Section 5.4) showed that two phases, before and after the 3rd-leaf stage, should be treated separately. It was at about the 3rd-leaf stage that the intermediate plant size was assessed in S3 of the field

Table 52. Path-coefficient analysis of shoot dry weight of 10 single-cross hybrids in S3 of the 1975 field experiment. The components are characteristics recorded during the field experiment.

Components	r_{simple}	*p* (values in italics) and *rp*					
		1	2	3	4	5	6
1 Kernel weight	+ 0.157	+ *0.413*	− 0.024	− 0.049	+ 0.044	− 0.204	− 0.021
2 Time to emergence S3	+ 0.276	+ 0.227	− *0.044*	− 0.026	− 0.203	+ 0.452	− 0.129
3 Number emerged plants S1	+ 0.248	− 0.157	+ 0.009	+ *0.129*	+ 0.046	+ 0.033	+ 0.189
4 Shoot dry weight ½(S1 + S2)/S3	− 0.000	+ 0.054	+ 0.026	+ 0.017	+ *0.340*	− 0.667	+ 0.239
5 Resistance to chlorosis	+ 0.625	− 0.077	− 0.018	+ 0.003	− 0.211	+ *1.089*	− 0.160
6 Shoot dry weight S6 (vigour)	+ 0.219	− 0.022	+ 0.014	+ 0.060	+ 0.203	− 0.436	+ *0.400*
R^2 = 0.855							

Table 53. Path-coefficient analysis of intermediate plant size of nine single-cross hybrids estimated on 18 May in S3 of the 1975 field experiment (data from Table 6). The data of the Components 1, 2, 3 and 4, and 5 are from Tables 1, 15, 13 and 24, respectively.

Components	r_{simple}	*p* (values in italics) and *rp*				
		1	2	3	4	5
1 Kernel weight	+ 0.568	− *0.148*	+ 0.006	− 0.000	+ 0.230	+ 0.479
2 Chilling resistance	− 0.333	+ 0.030	− *0.033*	− 0.010	+ 0.013	− 0.334
3 Time to emergence at 12 °C	− 0.398	+ 0.001	+ 0.017	+ *0.020*	− 0.197	− 0.239
4 Plumule dry weight at 12 °C	+ 0.835	− 0.094	− 0.001	− 0.010	+ *0.361*	+ 0.580
5 Initial shoot elongation rate 15/10 °C	+ 0.958	− 0.091	+ 0.014	− 0.006	+ 0.271	+ *0.771*
R^2 = 0.960						

experiment. This plant size did not correlate with shoot dry weight at the 6th-leaf stage ($r = -0.340$, Table 7). For those reasons, compound path analysis was carried out, first for intermediate plant size as a resultant variable, and second for (final) shoot dry weight as a resultant variable with intermediate plant size as one of the components. Most of the other components were chosen from trials carried out under controleed conditions.

The results of the path-coefficient analysis for intermediate plant size are given in Table 53. R^2 was extremely high; the variation of plant size was for 96 % determined by the components chosen. Initial shoot elongation rate had the highest path coefficient ($p = 0.771$) and also a very high simple correlation coefficient. The high simple correlation coefficient between plant size and plumule weight was composed of a rather low path coefficient and a strong indirect effect through initial shoot elongation rate. The other components had negligible direct effects. The data show that chilling resistance of just-germinated seedlings had no effect on plant size, although the temperature was very low from sowing until the date of plant size assessment (Figure 1).

Table 54 gives the data of shoot dry weight in the 6th-leaf stage. Leaf elongation rate had a low and non-significant simple correlation and negligible direct and indirect effects. The path coefficient of shoot dry weight in S6 was rather low. Intermediate plant size had a low and negative simple correlation coefficient, which was composed of a considerable positive direct effect ($p = +0.961$) and a stronger indirect effect through resistance to chlorosis. In this analysis, resistance to chlorosis shows again the strongest direct effect ($p = +1.608$).

The analysis shows that variation in plant size in the 3rd-leaf stage is mainly determined by initial shoot elongation rate and plumule size and that the major components of shoot dry weight in the 6th-leaf stage are plant size in the 3rd-leaf stage and chlorosis resistance.

Table 54. Path-coefficient analysis of shoot dry weight of nine single-cross hybrids in S3 of the 1975 field experiment (data from Table 4) with intermediate plant size (data from Table 6) as one of the components. The data of Components 2, 3 and 4 are from Tables 6, 24 and 4, respectively.

Components	r_{simple}	*p* (values in italics) and *rp*			
		1	2	3	4
1 Intermediate plant size	− 0.247	+ *0.961*	− 1.424	− 0.026	+ 0.242
2 Resistance to chlorosis	+ 0.591	− 0.851	+ *1.608*	− 0.011	− 0.154
3 Leaf elongation rate 15/10 °C	+ 0.373	+ 0.221	+ 0.160	− *0.116*	+ 0.106
4 Shoot dry weight S6 (vigour)	+ 0.361	+ 0.548	− 0.583	− 0.028	+ *0.425*
$R^2 = 0.823$					

8.5 Discussion and conclusions

The examples given in this chapter show that in some relationships the values of simple correlation coefficients and path coefficients were similar, and that in other relationships they were very different.

Path-coefficient analysis has yielded little new information about the effect of damage components before emergence (Table 52) or the effect of chilling resistance of just-germinated seedlings (Table 53) on seedling growth after emergence. Path-coefficient analysis has shown that resistance to chlorosis is a major component of shoot dry matter accumulation at low temperature (Tables 51, 53 and 54); simple correlation coefficients give the same indication.

Considerable differences between simple correlation coefficients and path coefficients were found in the following relationships. The simple correlation coefficient between shoot dry weight in the 15/10 °C treatment and initial shoot elongation rate was -0.276, whereas the path coefficient was $+0.925$ (Table 51). A similar situation occurred for the relationship between shoot dry weight in the field experiment and intermediate plant size ($r_{simple} = -0.247$ and $p = +0.961$) (Table 54). In both cases strong negative indirect effects via resistance to chlorosis masked the positive direct effect. The reason for the differences between simple correlation coefficients and path-coefficients was the coincidence of rapid inital growth and sensitivity to chlorosis in the same genotypes.

It appears that simple correlation coefficients may be misleading if there are strong indirect effects, particularly when those indirect effects are opposite to the direct effects. This implies that the choice of simple traits as selection criteria for complex plant characteristics, such as cold tolerance, should be based on simple correlation coefficients that reflect a direct effect.

9 General discussion and conclusions

9.1 Introduction

The aim of this study was to investigate what plant characteristics should be improved to promote maize production under cool temperate conditions. The investigations were confined to the seedling stage because it is at the beginning of the season that the adverse effects of low temperature are most significant. In autumn, when leaf area is at a maximum and a large amount of biomass is present, light is the main growth limiting factor (Struik & Deinum, 1982), whereas in this stage of development low temperatures in the range of 10 – 15 °C promote net assimilation by suppressing dark respiration (L. Sibma & W. Louwerse, personal communication). In spring and early summer the same temperature range results in plant damage and growth depression.

To investige what plant properties are involved in low temperature adaptation, two approaches are possible. A first approach consists of selection for the desired trait, e.g. high shoot dry weight at low temperature, followed by investigation of the plant characteristics that have changed simultaneously under the selection pressure. The second approach consists of a physiological analysis of existing genetic variation. We have followed the second approach. Genotypes that differed in seedling performance under cool field conditions were exposed to various low temperature treatments. In most experiments single-cross hybrids of a Dutch breeding company were used. A group of exotic accessions was also tested.

In this chapter, the main results of these investigations will be discussed; for most of the literature before 1981 we refer to Miedema (1982). A distinction is made between low temperature damage, before and after emergence, and retardation of growth processes in non-damaged seedlings.

9.2 Low-temperature damage before emergence

Types of low-temperature damage between sowing and emergence recorded in this study were: seed and seedling mortality after early sowing, chilling injury to seeds and seedlings, seedling malformations and reduced seedling vigour after long-term exposure to cool soil conditions.

Seed and seedling mortality in cold and wet soil has been a major constraint to maize growing in cool areas. This problem has for a long time dominated the research on cold tolerance. Formerly, the term 'cold tolerance' referred to seedling survival on-

ly. Nowadays a survival test is still called a 'cold test' a confusing term since it has become clear that seedling survival under cool conditions is primarily a question of resistance to soil pathogens that are active at temperatures around 8 – 10 °C, whereas germination and seedling growth are very slow in that temperature range. The resistance to soil fungi has turned out to be determined mainly by damage to the seed coat and not directly by genotype. Seed of high quality and the use of fungicide dressings have largely overcome the problem. See Bunting & Gunn (1974) for a review.

In all experiments of this study, seeds were dressed with fungicide. Seed and seedling mortality was only observed under extreme conditions. The very long period of 53 days between sowing and emergence in the first sowing of the 1975 field experiment, reported in Chapter 2, resulted in emergence ranging from 12 – 90 % in 10 single-cross hybrids. The average emergence was 54 % (Table 3). The pattern of genotypic differences did not occur in any of the cold treatments reported in Chapter 4, including a survival test with pathogenic soil. It seems likely that in the 1975 field experiment several adverse effects were involved, such as imbibitional chilling injury, effects of fungi, effects of lindane in the seed dressing and seedling malformations.

In the germination experiments with cv. Fronica, a 76-day exposure, in soil, to 8 °C resulted in 38 % survival, but at 6 °C survival was 85 % (Section 3.2). At 6 °C only one third of the seeds had germinated, whereas at 8 °C all seeds had, which presumably increased their sensitivity to soil fungi. This implies that selection for a lower minimum temperature for germination may result in increased seedling mortality by soil fungi.

A morphological aberration associated with low temperature was observed in the early sowings of the 1975 field experiment (Chapter 2). At least 15 % of the non-emerged seedlings had formed distorted shoots below soil level. Some other seedlings with distorted shoots did emerge, but with a folded first leaf. This disoriented growth was attributed to opening of the coleoptile before emergence by a faster growing first leaf. Such a dissimilar growth rate of coleoptile and first leaf was also observed at day/night temperatures of 18/6 °C but not at a continously low temperature of 12 °C (Section 3.3; see also Buckle & Grant, 1974). Physiological damage to seedlings or imbibed seeds occurs at temperatures below 5 °C. This so-called chilling injury received much attention in this study because it is regarded as a fundamental barrier to low-temperature adaptation of thermophilic plant species (Steponkus, 1981; Graham & Patterson, 1982; McWilliam, 1983).

Chilling resistance of just-germinated seedlings of the 10 single-cross hybrids was positively correlated with seedling performance under cold conditions after emergence, but not with survival or shoot growth rate before emergence (Table 16). However up to now, there is no clear evidence to support a causal relationship between chilling resistance of seedlings before emergence and plant characteristics involved in low-temperature adaptation (see also Subsection 7.3.3).

Long-term exposure to low temperature between sowing and emergence reduced seedling vigour after emergence. This after-effect was found in the 1975 field experiment where shoot dry weight of the first and second sowing lagged behind that of the

third sowing, even though the date of emergence was about the same (Table 5). A similar reduction of seedling growth after emergence was found in a pot experiment in which a pre-emergence cool treatment of four weeks outdoors was compared with a warm pre-emergence treatment of one week in a greenhouse (Section 5.3). A pre-emergence cool treatment of two weeks, however, did not reduce seedling growth after emergence.

To conclude, imbibed seeds and young seedlings may suffer from various adverse effects of low temperature. The type and severity of the injury depends on the temperature, the duration of the stress, the stage of seedling development and the genotype.

9.3 Low-temperature damage after emergence

After emergence, low temperatures may result in various types of damage, such as frost injury, chlorotic cross-bands and chlorosis (see Miedema, 1982).

Cold nights early in the season may cause frost injury if the temperature drops below -4 °C. The effect of freezing was not included in this study, however, because there was little evidence for genetic variation in this respect. Trials reported in Section 6.6 showed that cold nights with temperatures above freezing point, followed by non-stress day temperatures, did not visibly harm the plants. Tsjäpe (1972) reported that one cold night of 2 or 5 °C reduced stomatal aperture but hardly affected photosynthesis at 25 °C. Grzesiak et al. (1985), however, showed that prolonged exposure of maize seedlings to day/night temperatures of 25/5 °C reduced shoot growth in comparison with 25/15 °C. Versteeg (1985) observed that cold nights at high altitudes damaged maize plants which he attributed to excess of assimilates.

Cold nights in combination with low day temperatures may result in chlorotic cross-bands in the leaves (Table 44, Figure 17). Such cross-bands were also produced when maize seedlings were exposed to 4 °C in the dark for 3 days (Section 6.6). They are the result of chilling injury to leaf tissue just above the shoot meristem. The chlorotic tissue does not recover, but because it is a very small part of the leaf area its effect on plant photosynthesis is negligible.

A very common type of cold damage in maize is chlorosis. It occurs during long spells of cold and bright weather with day temperatures in the range of 7 – 15 °C. This type of chlorosis differs from the above-mentioned cross-bands in that it covers a larger part of the leaf area, it arises at higher temperatures, it depends on and increases with light intensity and the plants usually recover form chlorosis after a rise in temperature. Chlorosis is probably the most important damage of maize plants after emergence, but little quantitative data is available on its effect under field conditions. The present study shows that chlorosis may be associated with a reduction of dry-matter accumulation and leaf extension during and after the chlorosis-inducing conditions (Sections 6.4 and 6.5). Photosynthesis in chlorotic leaves is strongly reduced (Alberda, 1969; Bird et al., 1977), which will reduce seedling growth particularly after depletion of the seed reserves (Section 5.4).

Chlorosis is most pronounced in the temperature range of 10 – 15 °C; below 10 °C

leaf extension – and therefore production of chlorotic leaf area – is very slow. Green leaves were not visibly injured by short exposure to low temperatures, but exposure to 10 or 7 °C resulted in other types of injury: necrosis of leaves and shoot meristems, wilting and finally the death of the plants (Section 5.5). This wilting may be due to chilling injury of the stomatal movement system (Mustárdy et al., 1982, 1984).

A phenomenon not investigated in this study is photoinhibition, i.e. reduction of photosynthetic activity by high light intensity not accompanied by a decrease in chlorophyll concentration (see Powles, 1984, for a review). Photoinhibition caused by a combination of high light intensity and low temperature has been reported for maize (Long et al., 1983) and other thermophilic plant species (Powles et al., 1983).

In maize, photoinhibition of CO_2 assimilation was associated with a decrease of the variable chlorophyll-fluorescence emission of the leaves, which indicates that thylakoid membranes were damaged (Baker et al., 1983). This light-dependent injury may affect seedling growth in the field when low temperatures coincide with high light levels. Hetherington et al. (1983) showed a decrease of chlorophyll fluorescence of maize leaf sections kept at 0 °C in the dark. They found that this decrease was smaller in cold-tolerant than in cold-sensitive maize populations and suggested that measurements of chlorophyll fluorescence can be used for screening cold-tolerant genotypes.

The relationship between the kinetics of chlorophyll fluorescence and photosynthetic capacity is very complex, since differences in chlorophyll fluorescence may be the result of different types of injury to the photosynthetic apparatus. However, the recently developed technique of high-frequency light modulation enables a separation of chlorophyll-fluorescence quenching due to impairment of the photochemical and non-photochemical reactions of the photosynthetic process (Schreiber et al., 1986). With this technique, rapid screening of maize genotypes may be possible.

To conclude, low temperatures after emergence cause various types of damage to maize seedlings. Further investigations will elucidate the effects of damage to the photosynthetic apparatus on dry matter production under field conditions. The relationship between damage to the photosynthetic apparatus and leaf area development is still unclear, however. The increase of chlorosis and photoinhibition with light intensity may have a useful function because it protects the plants from excess of assimilates under conditions of slow growth.

9.4 Growth retardation at suboptimal temperatures above the injury threshold

The optimum temperature for growth processes in maize is around 30 °C. This applies to germination and shoot elongation before emergence (Section 3.2; Figures 3 and 4), to leaf appearance (Tollenaar et al., 1979; Warrington & Kanemasu, 1983), to leaf expansion (Duncan & Hesketh, 1968), and to shoot elongation and dry matter accumulation after emergence (Muldoon et al., 1984). For net photosynthesis Vong & Murata (1977) reported an optimum of 30 °C for maize plants grown at 30/25 °C (see also Duncan & Hesketh, 1968). However this optimum may be lower for plants acclimated to cool conditions (Bird et al., 1977; Blondon et al., 1981; Bennet et al.,

1982). The optimum for net photosynthesis also shifts to lower temperatures when measurements are done at low ligth intensities (Rainguez, 1979); this effect is attributed to the fact that dark respiration increases with increase of temperature, whereas gross photosynthesis did not increase due to light limitation. Optimum crop growth occurs in climates with mid-summer temperatures between 21 and 27 °C (Shaw, 1977). Therefore for the present discussion temperatures below that range will be considered suboptimal.

The minimum temperature for growth processes is in the range 5 – 15 °C. The minimum temperature for shoot growth before emergence is about 8 °C, which is above the threshold for physiological injury. Processes restricting heterotrophic shoot growth at 8 – 20 °C are probably cell division or cell extension and not mobilization or transport of nutrients (Christeller, 1984). The minimum temperature for shoot growth after emergence is below 7 °C (Section 5.5) and for net photosynthesis below 5 °C. (Long et al., 1983), but those temperatures cause considerable injury. The injury threshold for growth after emergence increases with light intensity and stress duration to about 13 – 15 °C, i.e. the minimum temperature for net chlorophyll accumulation. The question of what processes limit autotrophic growth has already been discussed by Miedema (1982). He argued that at suboptimal air temperatures above the injury threshold, leaf extension rather than net photosynthesis limits seedling growth. This agrees with data obtained for rice (Kishitani & Tsunoda, 1974) and data for temperate species that have been reviewed by Kemp (1984), who stated that 'temperature controls plant growth more through regulating the rates of reactions that utilize the products of photosynthesis than through direct effects on photosynthesis.' Rapid leaf elongation of seedlings results in an earlier closing of the canopy, which enables the crop to utilize the high daily inputs of solar energy early in the season.

Further experiments are required to test the significance of leaf elongation and photosynthesis for maize seedlings of different genotypes grown under cool field conditions.

9.5 Genetic variation and breeding

Genetic variation has been reported for several traits that may contribute to low temperature adaptation of maize. Main sources of cold tolerance are European flints and tropical highland accessions from Central and South America. The common breeding approach for low temperature adaptation comprises three steps:

- improvement of populations by cyclic selection and recombination
- development of inbred lines from these populations
- producing and testing of hybrids.

Population improvement is a time-consuming, but indispensible, step in the procedure. It aims at accumulating as many of the desired genes as possible and the exclusion of undesirable genes.

Screening for cold tolerance must be carried out at every step of the procedure. A

first question to be answered is: is it necessary to screen for all possible cold tolerance traits, for some main traits, or can the procedure be simplefied by concentrating the efforts on one basic trait.

Very little fundamental research has been done on the biochemical or physiological basis of genotypic variation of cold tolerance in maize. A few studies are worth noting. Mustárdy et al. (1982, 1984) exposed maize seedlings of a chilling-sensitive and a chilling-tolerant genotype to 8 °C and a light intensity of 80 W m^{-2} for various periods of time. Plants of the sentitive genotype wilted after 4 – 6 h, those of the tolerant genotype after 10 – 16 h of chilling. Wilting was associated with dysfunction of the stomatal closing system, which was attributed to chilling-induced damage to the chloroplasts of the guard cells. Stamp et al. (1983) investigated shoot growth, chlorophyll and carotenoid content, and the activity of the enzymes PEP carboxylase, RuBP carboxylase, NADP malate dehydrogenase and phosphofructokinase in seedlings of three cold-tolerant and three cold-sensitive inbred lines of maize that were exposed to temperatures ranging from 14/12 °C to 38/36 °C. A correlation matrix (not presented by Stamp et al.) of the data obtained at 14/12 °C showed that shoot dry weight correlated with phosphofructokinase activity but with none of the other traits. Chlorophyll content drastically decreased with a lowering of the temperature from 22/20 °C to 14/12 °C; at 14/12 °C the cold tolerant lines had a higher chlorophyll content than the sensitive lines. The carotenoid content increased with a lowering of temperature in the cold-tolerant genotypes but not in the cold-sensitive ones; at 14/12 °C a high correlation ($r = 0.965^{**}$) was found between chlorophyll and carotenoid content. Carotenoids protect chlorophyll from photo-oxidation (Anderson & Robertson, 1960. Mayfield et al, 1986). It seems likely that genotypic differences in chlorosis are caused by differences in carotenoid synthesis at low temperature. Further research is required to test this hypothesis on a large group of genotypes.

Another important study was carried out by Duncan & Hesketh (1968). They investigated the effects of temperatures ranging from 15/10 °C to 36/31 °C in glasshouses on 22 races of maize originating from various altitudes. At low temperature, the high-altitude races had higher leaf growth rates and dry weights at harvest than the low-altitude races but there was no difference in net leaf photosynthesis between the groups. Those data demonstrate that leaf growth rather than photosynthesis underlies genotypic differences in dry matter accumulation at low temperature. Absence of a relationship between net photosynthesis and growth at low temperature was also reported by Blondon et al. (1980) for a cold-tolerant flint and a cold-sensitive dent (corresponding with SH3 and SH6, respectively) and by Miedema & Sinnaeve (1980) for SH2 and SH10 (designated as F and D, respectively).

The data of the studies just discussed show that different mechanisms may be involved in genotypic variation in response to low temperature. Our study has shown that in the group of single-cross hybrids, several cold tolerance traits are statistically unrelated. In growth-chamber trials at 15/10 °C, no correlation was found between initial shoot elongation rate and elongation rate of the 3rd leaf (Section 5.2). In the 1975 field experiment, no correlations were found between survival in cold soil, time

to emergence and resistance to chlorosis (Table 7). Similar results were found by Pollmer (1969), who found very low correlations between germination at low temperature and chlorosis or growth at low temperatures in a group of 340 varieties. McConnel & Gardner (1979) showed that selection for improved germination at low temperature did not result in improved emergence or seedling vigour. Further selection experiments on separate traits are required to investigate whether functional relationships exist between cold tolerance traits. However the absence of strong statistical relationships between those traits, suggests that genetically and physiologically unrelated mechanisms are involved. If one basic principle plays a role, for instance damage to membranes of cells or cell organelles, many modifying factors infuence the ultimate plant response. In consequence, screening has to be done for several traits. The next questions to be answered are then: what traits of the cold tolerance complex are important; and what is the most effective screening technique?

In his review paper Miedema (1982, p. 120 – 122) pointed out that about ten different traits contribute to survival and growth of maize seedlings at low temperature. In a cool maritime climate, resistance to chlorosis and leaf extension were supposed to be the most important. Path-coefficient analysis (Chapter 8) has shown that rapid initial growth is also an important component. A critical phase in shoot dry matter accumulation was found at about the 3rd-leaf stage, when seed reserves are depleted and carbohydrate supply has become completely dependent on photosynthesis. Differences between two genotypes suggested a relationship between this growth crisis and sensitivity to chlorosis. Further research on this subject with a larger group of genotypes is required.

Screening for cold tolerance can successfully be carried out under cool field conditions or in cooled greenhouses. Screening in artificially-lit growth-chambers may be less suitable because of space limitations, site effects and light intensities that are often too low. Screening for chlorosis resistance is rather simple as the expression of this trait depends on the environmental conditions only. Growth at low temperature is more difficult to screen for since it is the result of specific genotype-temperature interactions and plant vigour independent of temperature. Further information on various screening techniques is described by Miedema (1982).

To improve low temperature adaptation in maize, plant breeders use existing genetic variation. The possibility of improvement through induced mutations, by seed treatment or cell culture, can be neglected because the desired characteristics are manifold and polygenically inherited. Recombination breeding has been proven successful for seedling performance under cool field conditions (Dolstra & Miedema, 1986). Maize has, in general, a great adaptability to various environments. The ultimate possibilities of adaptation to low temperature are hard to predict. Systematic breeding research utilizing genetically broad material will provide the answer.

Summary

This report describes investigations of what selection criteria and screening techniques should be used for the improvement of cold tolerance in maize. A group of ten single-cross hybrids with differential cold tolerance was analysed for their response to low temperature under field, greenhouse and growth-chamber conditions. For some experiments varieties were used and in others a group of exotic accessions. The experiments focus on early vegetative growth from germination until the appearance of the sixth leaf. In the analysis of plant response a distinction was made between low-temperature damage and the effects of sub-optimal temperatures above the injury treshold. A further distinction was made between effects before and after emergence.

A long period of cold weather between sowing and emergence resulted in various types of damage: reduced survival due to seed rot and seedling malformations; delayed time of emergence; and a negative after-effect on seedling vigour after emergence. Under laboratory conditions a 6-day exposure of germinated seedlings to 2 °C resulted in chilling injury. After emergence, low day temperatures in the range of 10 – 15 °C retarded or inhibited the greening of young leaf tissue, which resulted in chlorosis. Chlorosis was promoted by high light intensities. The proportion of chlorotic leaf tissue increased with leaf extension under the stress conditions.

Before emergence, the threshold temperature for physiological damage is about 5 °C, whereas the minimum temperatures for germination and shoot growth are in the range of 6 – 8 °C. After emergence the minimum temperature for shoot growth was about 7 °C, but the threshold temperature for injury (chlorosis) was much higher, i.e. 10 – 15 °C, due to the influence of light; cold nights of 4 °C did not visibly harm the plants. Growth processes, such as emergence, leaf extension and dry matter accumulation, were strongly retarded at temperatures below about 12 °C.

Genotypes differed in the various traits expected to contribute to cold tolerance. The differences in low-temperature damage were more pronounced than growth differences of non-damaged plants. Most of the cold-tolerance traits did not significantly correlate with each other, which suggests that separate processes are involved. Path-coefficient analysis showed that resistance to chlorosis was a major component for shoot dry matter accumulation at low temperature, even if little chlorosis was observed. Rapid leaf extension is an important trait as it increases productivity by closing the canopy sooner. Screening can be done for seperate processes or for the whole complex of traits in the field or in cooled greenhouses.

To conclude, some recent literature data on physiological backgrounds of cold tolerance are discussed.

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