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How to maintain improved cultivars

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Abstract Improved cultivars loose their identity and healthiness unless maintained properly. Contaminating and degrading forces, such as outcrossing, volunteer plants, mixing, natural selection, mutation and seed-borne diseases, are at the root of this. Maintenance selection can prevent this deterioration. How it is carried out depends on the reproduction system of the crop. Crops are therefore classified into four categories; typical cross-pollinating crops, self-pollinating crops with a substantial amount of outcrossing, typical self-pollinating crops with little outcrossing, and the vegetatively reproduced crops.

Generally some of the "breeder seed" is used to plant a small plot with spaced plants. A fair number of healthy plants of the cultivar type is selected and the seed is harvested per plant. The progenies of the selected plants are grown in small plots. Non-uniform or deviating plots and plots with a seed-borne disease are removed. The seed of the progenies that are healthy, uniform and similar (and so of the cultivar type) are harvested per progeny to be tested next season on larger plots. The same selection is applied and only the seed of the progenies that are healthy, uniform and similar are harvested together to produce the "breeder seed". The details of this maintenance selection vary with the reproduction system, the multiplication rate of the crop and the possibilities available to the breeder. Seven crops, potato, common bean, barley, wheat, faba bean, quinoa and maize are discussed here as they represent the different reproduction systems and multiplication rates, while being important Andean food crops.

Keywords Breeder seed · Basic seed · Certified seed · Contamination · Local cultivar · Mixing · Maintenance selection · Multiplication rate · Mutation, Natural selection · Reproduction system · Seed-borne disease

Introduction

Plant breeding results in the production of improved cultivars. These cultivars are usually considerably more uniform than the local cultivars grown and maintained by the farmers (see below). The improved cultivars have to be multiplied to reach the farmers. Multiplication is a repeated process as seed should be available at the start of each growing season. Each multiplication cycle starts from the stock seed of the cultivar, the "breeder seed" (BS). This BS has to remain true-to-type and healthy. During the whole process of maintenance and multiplication, contamination and even complete loss of the improved features of the cultivar can occur (Bateman, 1947; Hanson, 1973; Kelly, 1988; Laverack, 1994; Kimmich, 1996). Prevention of this contamination is most effective at the basis of the process, at the maintenance of the cultivar.

The way crops reproduce determine to a large extent their genetic make up and how breeders should improve

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and maintain them. In this respect crops can be classified into four categories: (1) typical cross-pollinating crops, (2) self-pollinating crops with a substantial amount of outcrossing, (3)typical self-pollinating crops with very little outcrossing, and (4) the vegetatively reproduced crops.

Improved open pollinated cultivars of the first category, such as maize, *Zea mays*, are genetically narrowed populations, with high frequencies of the desired genes. They are hard to maintain. Improved cultivars of crops of category 2, like quinoa, *Chenopodium quinoa*, and faba bean, *Vicia faba*, consist of similar desirable genotypes, but maintaining them in this composition is far from easy. Improved cultivars of crops of category 3, like wheat, *Triticum aestivum*, barley, *Hordeum vulgare* and common bean, *Phaseolus vulgaris*, consist of very similar desirable genotypes, and maintaining them in this composition is fairly simple. Improved cultivars of crops of the last category, such as potato, *Solanum tuberosum*, consist of a single genotype, a clone. Its genetic purity is easily maintained, but to keep it free from pathogens, especially viruses, is very difficult.

Improved cultivars remain improved only when proper maintenance procedures are applied. Especially for crops with large acreages and low multiplication rates (seed yield/seed rate) such as potato, common bean, barley and wheat, the most important approach to keep cultivars healthy and true-to-type consists of various forms of maintenance selection.

Despite the importance of maintenance selection, very little has been published about it. About the multiplication, the seed production, much more information is available. Therefore the basic principles of maintenance selection are discussed here, while the multiplication aspects are mentioned only summarily. As maintenance selection and seed production are very important for developing regions (Hrabrovsky, 1982; Feistritzer, 1982) the maintenance selection has been discussed here with such a region, the Andes, in mind.

The local cultivars

Before formal plant breeding developed, many local cultivars existed in all crops. They developed over time as the combined result of human selection, natural selection and gene exchange within and between local cultivars (see contaminating factors below). These cultivars, landraces, were almost always highly variable. In modern agriculture the landraces have been replaced by improved cultivars produced by conscious selection and adapted to agriculture based on external inputs. In the Andean regions the local cultivars of the food crops are usually of the landrace type. Even recently introduced entities from elsewhere tend to become landraces after some time.

Landraces have a certain identity, suggesting that the farmers know how to maintain that identity. Parlevliet (2003) concluded that landraces tend to get their apparent identity from a few easily recognizable traits such as earliness and seed type, and despite considerable variation for many other characteristics.

Farmers usually maintain their local cultivars by withholding part of their harvest for the next growing season (Friis-Hansen, 1996). This often involves no selection at all. If selection is applied it will be some form of mass selection. The farmer selects seed from his own field, either before or after harvest, the selection being based on either the whole plant or the economic part of it (the kernel or the ear in maize for instance).

What happens to improved cultivars when they are released to farmers, who will maintain these cultivars themselves? These cultivars will be grown surrounded by local cultivars. Contamination in such a situation is difficult to prevent, especially in the case of cross-pollinating crops. They tend to loose their genetic advantage over the years unless maintained and multiplied in a proper way to protect them from becoming contaminated.

The contaminating and degrading forces

The contaminating forces vary in importance and intensity with the crop. These forces, directly or indirectly, enlarge the genetic variation within the cultivars with new genotypes of a usually lesser agricultural potential (Bateman, 1947; Humphreys, 1982; Laverack, 1994; Kimmich, 1996). The forces are:

(1) **Outcrossing** to local cultivars. Cross-pollinating crops such as maize, but also quinoa and faba beans, with a substantial outcrossing percentage and even typical self-pollinating crops like wheat, barley and common bean show a low percentage of outcrossing. After outcrossing the new genotypes recombine and segregate into a range of new genotypes enlarging the genetic variation of the contaminated cultivar.

(2) **Volunteer plants**. If the cultivar to be maintained is sown on a plot, that carried another cultivar of the same crop not too long ago, seedlings of that former crop may grow up together with the seedlings of the cultivar to be maintained and will be harvested together, enlarging the genetic variation with these impurities (Kelly, 1989; Laverack, 1994; Kimmich, 1996).
(3) **Mixture**. From harvest to the next planting mixing with seed from another cultivar of the same crop may occur. Any handling such as sowing, harvesting, threshing, cleaning, storage, bagging may cause mixture (Laverack, 1994; Kimmich, 1996). The result is the same as under 2).
(4) **Mutation**: During maintenance mutations may and do occur, whereby it should be taken into account that the majority of the mutations are of a negative nature, reducing the agronomic value of the carrier. Since mutations are relatively rare this factor is probably the least important of the degrading factors mentioned.
(5) **Natural selection** is, especially in cross-pollinating crops, of considerable importance (Johnson & Haigh, 1966; Snaydon, 1978; Taylor et al., 1990). The released cultivar nearly always exists of different, usually similar, genotypes. These different genotypes within a cultivar will never produce propagules (seed, tubers) in equal amounts and with equal fitness. As a result the frequencies of the different genotypes tend to change over time. The genotypes favoured by natural selection are often not the genotypes favoured by the farmer.
(6) **Seed-borne pathogens,** loose and covered smut in cereals, viruses in tuber crops, anthracnose in beans, can spread easily if not contained. Apart from their negative effects on the yield and on the quality of the harvested propagules (increased proportions of infected propagules) they also affect the genotypic composition of the cultivar as the affected plants produce far less propagules than the healthy plants.

The above mentioned forces result collectively in an enhanced and changed genetic variation, albeit not very significant on a yearly basis. These changes accumulate over the years until the genetic potential has decreased and the genetic variation has increased to the level of that of the surrounding local cultivars. When no specific measures are taken, the accumulated effects can be considerable, reducing the genetic gain realised by the breeding to almost zero within several years.

Maintenance of a cultivar

In order to release an improved cultivar it has to be multiplied, a process that is repeated during the cultivar's lifetime. Each multiplication cycle has to start from its basic stock seed, breeder seed, (Fig. 1). This BS has to be protected from the contaminating forces, mentioned above.

The best way to maintain a cultivar is to store a sufficient amount of seed of that cultivar under conditions (usually very low temperatures) that keep the seed viable for a long time (Kelly, 1988; Laverack, 1994). The amount of stored seed should be sufficient to start many multiplication cycles. This asks for a huge storage space for crops with low multiplication rates and relatively large seed size such as common bean, faba bean, barley and wheat. For such crops this is not a feasible solution. And if such storage is not possible,

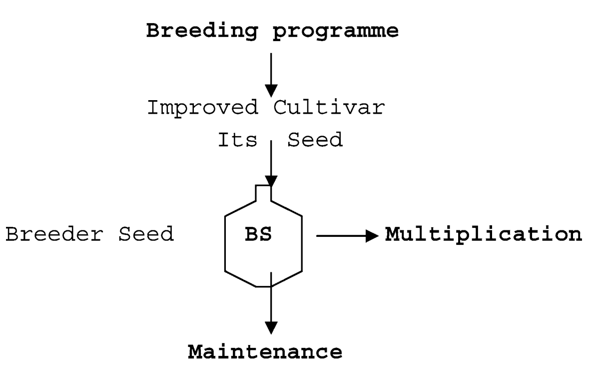


Fig. 1 Scheme from breeding to maintenance and multiplication

maintenance selection is the proper way to maintain a cultivar.

To maintain a cultivar true-to-type and healthy, roguing (negative mass selection) is an imprecise method (Laverack & Turner, 1995). Only through judging the progenies of individual plants, ears, pods, panicles, it is possible to assess the genetic purity of seed stocks (Slootmaker et al., 1982; Drijfhout, 1982; Mastenbroek, 1982; Kelly, 1988; Laverack, 1994; Kimmich, 1996).

Maintenance selection is in fact an extension of the normal breeding process (Slootmaker et al., 1982). The difference is that the selection is relatively mild and that the aim is not improvement, but "keeping the identity unchanged". The identity is determined by the concentration of genotypes (narrowed genetic variation) around a certain, desired plant type. So the selection should maintain both that plant type and the relative uniformity. Another aim of the selection is keeping the cultivar free of diseases.

The end product of the breeding is a "bag" of seed of the cultivar. That seed constitutes the *"Breeder Seed"*, the basis from where the *multiplication* and the next *maintenance* is started. The maintenance of BS is normally the responsibility of the breeding department of the organization. The multiplication is usually carried out by another unit of the organization, the production department (Kelly, 1988; Laverack, 1994).

The maintenance selection based on progeny testing is basically the same for self-pollinating crops, self-pollinating crops with a substantial amount of outcrossing and vegetatively reproduced crops and can be treated together (group I). The cross-pollinating crops (group II) follow slightly different procedures and are subdivided into crops where the important characteristics are assessed before flowering (forage crops, cabbages, carrots, onions) and into crops that are assessed after flowering, such as maize and rye.

As the plants and the progenies have to be assessed carefully it is essential that the site at which the maintenance selection is carried out is highly uniform. The uniformity can be greatly increased by thorough soil preparation, fertilizing it well and by the application of irrigation if needed. In short, by using optimal growing conditions. This has an additional advantage. The seed production is much higher, which is of great importance for crops with low multiplication rates. The multiplication rate can be further enhanced by using a lower seed rate than normally used by the farmers. This reduced plant density has an additional advantage. It is easier to discern and so to rogue deviating plants. It is also essential that the field is free of volunteer plants of the crop to which the cultivar to be maintained belongs.

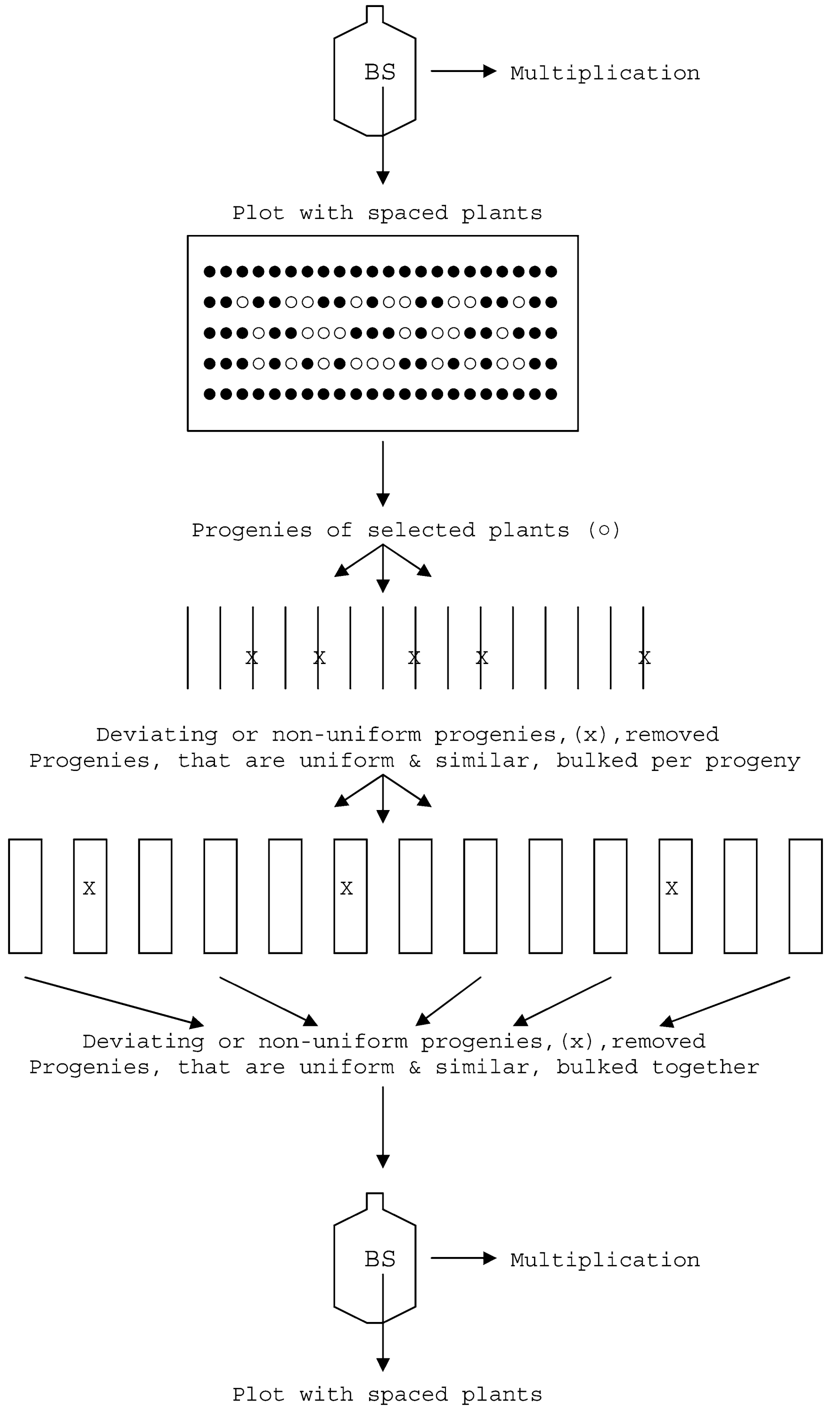
Maintenance selection group I

The maintenance selection (Fig. 2) starts with a small plot containing a number of spaced plants, derived from the BS as described by Drijfhout (1982) for beans. The plants must be well spaced to allow for individual plant assessment and for the harvest of sufficient seed per plant, especially important for crops with a low multiplication rate such as potato, common bean, faba bean, barley and wheat. A fair number of healthy plants of the cultivar type is selected and marked for progeny testing. Plants with a seed-borne disease are removed as soon as they can be identified. The seeds of the marked plants are harvested per plant and sown in small plots the next season, the first cycle progenies.

The progenies are assessed several times during the season. Progenies of plants, genotypically of the cultivar type, should resemble each other and be relatively uniform. Therefore all progenies that deviate in one or other way from the average type or are too heterogeneous are discarded. Only progenies that have the required uniformity and are of the same type are selected and the seed is bulked per progeny. If in a progeny only one or two plants deviate phenotypically, including being infected by a seed-borne pathogen, the whole progeny should be discarded.

There is now a much greater amount of seed per progeny to repeat this process with much larger plots, the second cycle progenies. The selection is exactly the same, but if the selection in the first cycle was carried out well, far fewer progenies will be removed in this second cycle. The seed harvested from the second cycle progenies with the required uniformity and plant type is now bulked together to produce BS. For the next cycle of maintenance one uses some of the selected second cycle progenies. From these progenies seed from individual plants, ears, pod or panicles is harvested to form the first cycle progenies the next season. The end product of each second cycle of progeny testing is BS to start the multiplication from and seed of a number of individual plants, ears, pods or panicles to start the next first cycle progenies.

Fig. 2 Maintenance selection, general scheme, starting from the bag of Breeder Seed (BS)



Maintenance selection group II

In cross-pollinating crops cultivars cannot be maintained unchanged for a prolonged period, unless the seed is stored under optimal conditions (Julén, 1982). When maintenance selection is practised the cultivar is bound to change genetically, either in a negative or in a positive way. Which way the cultivar will go depends on the balance between the contaminating and degrading forces on one hand and the selection pressure against these forces on the other hand.

An improved cultivar is a gene pool where the genes are reshuffled into a new set of genotypes each generation. Contamination through outcrossing, mixing, volunteer plants, natural selection and mutation all will change that gene pool for the worse. Measures, which reduce the risks of these negative forces, together with a proper maintenance selection can neutralize these negative effects on the gene pool or even turn them into positive ones by selecting sufficiently strong. This is what breeders of open pollinated crops usually do. They select stronger than necessary to ensure a gradual improvement of the cultivar. After each cycle of maintenance selection the BS is in fact a slight improvement over the former one. Repeated maintenance selection results in a continued improvement over time provided the number of progenies are always kept fairly large (Fig. 3).

However, the cross-pollinating crops have to be dealt with differently depending on whether the progenies are assessed before or after flowering. In the latter case pollination by undesirable plants cannot be prevented. A second difference is that the characteristics of crops to be assessed before flowering are usually characteristics related to the vegetative growth complex. Selection for increased yields of such characteristics tends to be negatively associated with characteristics related to the generative growth complex, i.e. seed yield (Johnson & Haigh, 1966; Julén, 1982; Snaydon, 1978). Due to this negative association a fairly strong natural selection tends to occur in the seed production phases. In spinach, *Spinacia oleracea*, leaf yield is positively associated with late bolting, and negatively with seed yield, which results in a strong natural selection towards earlier bolting during the maintenance and seed production of late spinach cultivars. The late cultivar Noorman, maintained and multiplied by six breeding firms, appeared to differ considerably in earliness, and so in leaf yield, between firms, from early-mid season to very late (Parlevliet, 1967). In Brussels sprouts Johnson and Haigh (1966) observed that a fairly strong selection was needed to maintain the agricultural value of the cultivars investigated. In forage crops the general advice is to maintain the cultivars in the region of its development as maintenance in another region and therefore under different environmental conditions may result in undesirable shifts of the cultivars due to strong natural selection (Snaydon, 1978; Julén, 1982; Taylor et al., 1990).

When the assessment is done before flowering the selection intensity should be fairly strong to counteract the natural selection. Relatively many progenies have to be removed and also within progenies selection for the right type of plants can be done. When the assessment is done after flowering as with maize it is advisable to exclude the pollination by the progenies to be discarded. This is possible by using the remnant seed approach. Maize has a fairly high multiplication rate and per selected ear only a small part is sown in the first progeny cycle. Of the selected progenies the remnant seed is used to plant the second progeny cycle. As there is still sufficient seed the plot can be larger in this second cycle. To allow for a fairly strong selection the number of plants (ears) to start from should be fairly large.

Discussion

The crops mentioned in Table 1 represent the various modes of reproduction. The approaches discussed here are guide-lines. The actual approaches of individual breeders usually differ depending on the circumstances they have to cope with. The size of the maintenance programme depends on the amount of breeder seed needed, the seed size, the multiplication rate and the selection intensity expected to be carried out, being much larger for faba beans and quinoa than for common beans, barley and wheat.

The multiplication rate, the quantity of seed produced per unit of seed sown, plays an important role. These rates differ greatly, especially with crop species (Table 1) but also with the cultivar and with the growing conditions such as input level and spacing. Within each crop, seed size varies considerably with the cultivar (up to threefold). The larger the seed the lower the multiplication rate. When lower seed rates and wider plant spacings are combined with optimal growing conditions the multiplication rates can increase considerably. The data in Table 1 are therefore of an indicative nature.

Table 2 gives an idea of the area with second cycle progenies (excluding the removed progenies) needed to produce sufficient BS to plant a multiplication field of two ha. This area increases with larger seeds and lower multiplication rates. Potato, with very large

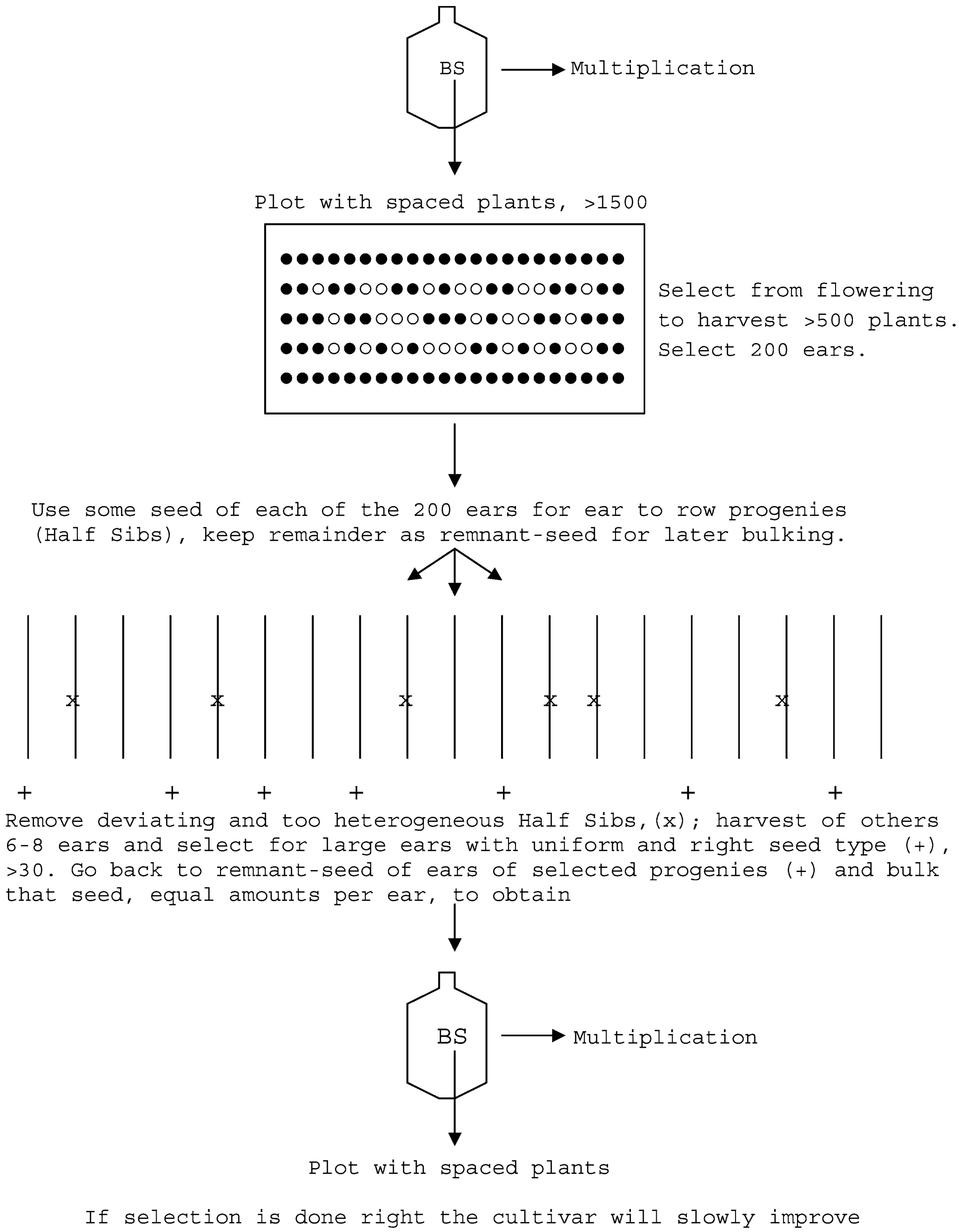


Fig. 3 Maintenance selection of a maize cultivar

Table 1 Multiplication rates of seven Andean food crops without and with optimal inputs and their reproduction systems

Crop	Without inputs	With inputs	Reproduction system	% Outcrossing
Potato	2–4	>10	Vegetatively	–
Common beans	4–7	>15	Self-pollinating	<1[a]
Faba beans	3–6	>12	Self-pollinating	20–40[b]
Barley	6–10	>25	Self-pollinating	<2[c]
Wheat	7–12	>25	Self-pollinating	<3[d]
Maize	25–50	>120	Cross-pollinating	>95
Quinoa	60–120	>300	Self-pollinating	10–25[e]

[a] Drijfhout (1982)
[b] Bond and Poulsen (1983)
[c] Wagner and Allard (1991)
[d] Martin (1991)
[e] Galway (1989)

Table 2 Area in m² per cultivar to be maintained needed to produce the amount of BS in kg sufficient to plant 2 ha of multiplication (BS for 2 ha, kg) and number of ha that can be planted with the yield from the 2 ha of multiplication (Ha)

Crop	Area in m²	BS for 2 ha,kg	Ha
Potato	1500	3000	25
Barley/wheat	650	200	50
Common bean	700	180	30
Faba bean	1000	180	30
Quinoa	60	20	650
Maize	150	60	250

Table 3 Number of spaced plants planted, number of spaced plants selected for progeny selection, number of progenies selected in the first progeny cycle and number of progenies selected in the second progeny cycle of a cultivar to be maintained

	Plot sp. pl.		Progenies selected	
Crop	Planted	Selected	1st cycle	2nd cycle
Potato	>500	>250	>150	>100
Barley/wheat	>1000	>250	>150	>100
Common bean	>250	>100	>80	>60
Faba bean	>1000	>300	>120	>60
Quinoa	>300	>50	>10	>5

propagules and a very low multiplication rate require about 1500 m² to produce the several tons of BS needed to plant the 2 ha, which would produce a yield of seed potatoes sufficient for only some 25 ha. For the small seeded quinoa with a high reproduction rate only 60 m² is needed to produce the 20 kg of BS required to plant the multiplication field of 2 ha, which could produce seed to plant some 650 ha (Table 2). The data presented are indications.

The number of plants and progenies planted and selected in the various stages of a modest maintenance selection programme, as suggested in Table 3, vary considerably. These variations are primarily due to differences in cross pollination rates; more cross pollination requires a stronger selection and so more plants to start with. High multiplication rates, on the contrary, reduce the number of plants one can start with as is shown by quinoa.

If the seed is stored under very dry conditions it is possible to keep it viable for a few years. In such cases it is advisable to alternate a cycle of maintenance selection with such a storage period. It is cheaper and the chances of deterioration are further reduced.

In typical self-pollinating crops the contamination rate due to unwanted cross-pollination varies from low (wheat and barley) to very low (common bean). But it cannot be ignored completely. Significant in the deterioration of cultivars are some seed-borne diseases, such as loose and covered smut in wheat and barley, and anthracnose in beans. Selection for healthy plants and seed therefore is important. Because of the low reproduction rate several cycles of maintenance and multiplication are required. The smaller number of plants started with and the milder selection applied on average in a bean cultivar compared with barley and wheat is due to its lower cross-pollinating rate (Table 1) and the fact that its plant type is more easily assessed in the field. In this group of crops maintenance selection is quite effective as Zeven (1980) showed. He compared the BS of the barley cultivar Zephyr maintained for 12 years by three breeders. The three stocks were still the same.

Self-pollinating crops with a substantial rate of outcrossing like quinoa and faba bean (Table 1) can be treated as self-pollinating crops, but require a strong selection for uniformity within and similarity between the progenies. The reproduction rates differ strongly, being high for quinoa and low for faba beans (Table 1). The low reproduction rate of faba beans implies that the requirements for the selection during maintenance and multiplication must be adhered to even more strictly than with quinoa. The plant structure of quinoa allows bagging to enforce self-pollination, which can help to prevent outcrossing during the maintenance programme.

In vegetatively reproduced crops such as potato the maintenance of the identity and uniformity usually is easy (Mastenbroek, 1982). The main problem is formed by accumulation of seed-borne pathogens present in or on the plant parts used for the reproduction, tubers in the case of potato (many viruses, a few nematodes and fungi). Modern in-vitro techniques circumvent this problem, while they also increase the multiplication rate considerably. The in-vitro approach would be ideal for maintaining potato cultivars, but it is expensive. The other, more conservative approach, maintenance selection, is not cheap either. This is mainly due to the low multiplication rate and the large propagule size of the potato (Table 1). Because of this, both maintenance and multiplication require several generations during which seed-borne diseases can accumulate. To protect the progenies from new viral infections, insecticides to control aphids could be applied or the maintenance could be carried out in areas with low aphid pressures such as areas well above 3000 m in the Andean regions (Thiele, 1999).

Multiplication

The multiplication stage is started from the breeder seed. Depending on the demand for seed of an improved cultivar and its multiplication rate it may be necessary to have more than one multiplication cycle to produce sufficient commercial seed. This is usually the case with crops with a low multiplication rate like potato, faba bean, common bean, barley and wheat. This must be taken into account.

The seed of the last multiplication cycle and so the seed to be commercialized is described as *certified seed*. In crops with very high multiplication rates, such as lettuce, the certified seed is directly derived from breeder seed. When there are two multiplication cycles, the seed produced from the first cycle is called *basic seed*, that of the second cycle certified seed, which can be sold. Sometimes there are more than two cycles (Hanson, 1973). It is than allowed to have two cycles of basic seed, *pre-basic seed* and basic seed. Also certified seed may be multiplied again into certified seed (*certified seed first* and *second* cycle). But each stage has to meet certain uniformity standards, which differ somewhat among countries.

At each cycle of multiplication the same contaminating and degrading forces will operate as during the maintenance selection. During the multiplication it is possible to remove off-type plants in order to meet the uniformity standards. This is a relatively costly activity and if there are too many impurities roguing is ineffective (Kelly, 1988; Laverack & Turner, 1995). When outcrossing is an important factor (maize, quinoa, faba bean) the multiplication field should be planted sufficiently far from other fields of the same crop or planted at such a time that its flowering does not coincide with the surrounding local cultivars. The field too should be screened for volunteers. In case of seed-borne diseases affected plants should be removed as early as possible.

Conclusions

The best way to maintain cultivars is storing breeder seed under optimal conditions in amounts sufficient to last for several years. When this is not possible or economical the cultivars should be maintained through forms of progeny selection to neutralize the effects of the various contaminating and degrading forces.

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