CHAPTER 1

Crop domestication and the first plant breeders

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1.1 INTRODUCTION

If the story of modern humans from the beginning to the present day could be compressed into a feature-length movie, the era of crop domestication would occupy a scene approximately six minutes long, starting about ten minutes from the movie's end. During that scene, the action would be scattered and sporadic; the domestication of any individual crop species would almost always occur in only a single locality and occupy only about 15 seconds to 2 minutes of the film.

In that brief era, in those rare places where today's crops were born, every farmer was a plant breeder. And through succeeding millennia, as agriculture spread across the surface of the planet, much of settled humanity came to participate in plant breeding.

Studies of ancient artefacts and botanical remains, ancient DNA, phytoliths, living plant populations, and the agricultural practices of surviving indigenous societies have converged to provide us with a vivid but still incomplete account of the first plant breeders' genetic revolution. Conventional wisdom based on those studies tells us that domestication was preceded by a period of archaic cultivation, during which people encouraged the growth of particular species and harvested their seed or other plant parts; that when people began to sow a portion of their harvested seed, they selected—automatically and unconsciously—for genes of domestication, such as those curtailing seed dispersal and dormancy; and that, as our ancestors developed a mutual dependency with domesticated plants, they became intentional and versatile plant breeders, selecting for a wide range of desired traits in species grown for grains, roots, tubers, fruits, vegetables or fodder.

Conventional wisdom usually gains its status by being accurate in its generalities but off the mark in some of its specifics. As we will see, that is the case with crop domestication. My purpose in this chapter is not to summarize the 'where' and 'when' of domestication, species by species, nor is it to analyse theories on the origins of agriculture. Those tasks would entail the boiling-down, if not the over-cooking, of a vast and fascinating literature (e.g. see Zeder *et al*., 2006; Sauer, 1993; and Harris and Hillman, 1989). Rather than attempt to summarize that literature, I briefly tabulated in Table 1.1 what is believed to be true, both geographically and chronologically, about the domestication of today's major crops.

Keeping in mind that humanity's brief experiment with domestication involved people in every quadrant of the globe, I will concentrate on the 'how' and 'why' of domestication, on questions about the first plant breeders themselves and the species they transformed: Why did they domesticate some species and not others? How did their farming practices change gene frequencies in plant populations? How long did domestication take? Why did people select for particular traits: unconsciously, intentionally or indirectly? How did their actions affect the genetic structure and diversity of today's crop species? And, finally, what kinds of skills and knowledge did they pass down to the farmer-breeders of more recent times?

Any effort to answer those questions must draw upon examples from the available literature, in which today's major crops, largely cereals and grain legumes, feature most prominently. Although no set of examples can represent the full geographical and botanical range of domestication, I have attempted to rely upon those people,

TABLE 1.1
Species domesticated in each of eight world regions, with approximate age of the oldest evidence of domestication

Region	Species	Common name	Age of the oldest evidence of domestication (years BPE)
West Asia	*Hordeum vulgare*	Barley	10 500
	Triticum turgidum	Emmer Wheat	10 500
	Cicer arietinum	Chickpea	9 500
Africa	*Sorghum bicolor*	Sorghum	8 000[a]
	Pennisetum glaucum	Pearl Millet	?(1)
Eurasia	*Brassica campestris*	Rape	3 500
East Asia	*Oryza sativa*	Rice	7 000
	Glycine max	Soybean	4 000
New Guinea	*Musa* spp.	Banana	7 000(2)
	Saccharum officinarum	Sugar Cane	?
South America	*Ipomoea batatas*	Sweet Potato	4 500
	Arachis hypogaea	Groundnut	4 500
	Solanum tuberosum	Potato	4 500
	Manihot esculenta	Cassava	4 500
	Phaseolus vulgaris(3)	Common Bean	7 500
Mesoamerica	*Zea mays*	Maize	7 500
	Gossypium hirsutum	Cotton	7 500
North America	*Helianthus annuus*	Sunflower	3 000

NOTES: (1) Wendorf *et al.* (1992) found archaeological evidence that wild millet and sorghum were being used in the Sahel 8000 years before present. The sorghum specimens showed evidence that they were in the process of domestication. (2) Denham *et al.,* 2003. (3) Independently domesticated in Mesoamerica as well. Species listed are among the world's 20 most widely grown crops, on a land-area basis (FAO, 2005). Information is from Sauer (1993) unless otherwise indicated.

places and plants that best illustrate the important features of domestication.

1.2 SELECTION AMONG SPECIES

There is little doubt that certain species were 'pre-adapted' (Zohary, 1984) for domestication. Either entire populations or individual plants within populations had to attract the attention of humans before they could be manipulated. With exceptions, plants or populations that exhibited unusually large or numerous edible parts; self-pollination (in sexually propagated species); ease of propagation (in vegetatively propagated species); or delayed seed dispersal (e.g. chickpea: Ladizinsky, 1979) caught the eyes of early cultivators. Bar-Yosef and Kislev (1989) listed characteristics of certain wild cereals (relative to other wild plant species) that attracted early west Asian domesticators: larger grain, local abundance, annuality, lower seed dormancy, diploidy, harvestability and relative ease of seed dehulling.

A common characteristic among crop ancestors was their weediness: their tendency to thrive in disturbed, fertile soils like those associated with human habitation. The circumstances of domestication are, of course, different for every species. In some places, people started out by harvesting conveniently large stands of annual grasses; in others, variations on the so-called 'rubbish heap' theme were at work (Hawkes, 1969). Many crop ancestors were just as responsible for seeking out humans and human-made environments as were people for tracking down the plants. Indeed, according to Hawkes (1969), it "must have seemed little short of miraculous to find that plants needed for food sprang up by their very huts and paths".

In west Asia, however, those destined to become the first agriculturists tended to make their homes near reliable water sources, whereas they gathered wild grains from stands that were often some distance away (Willcox, 2005). Also relying on the west Asian domestication experience, Abbo *et al*. (2005) labelled the rubbish-heap hypothesis 'environmental determinism' that "tends to underestimate the role of human initiative in the Neolithic transition".

One thing is certain: the original domesticators did not adopt just any species that showed up at their doorstep. Then, as now, people had strong ideas about the usefulness of some plant species and the unacceptability of others. Plants with the most to offer were domesticated long ago, while others that were sufficiently weedy, but less desirable, repeatedly presented themselves to humans, only to be ignored or targeted for eradication (Hawkes, 1969).

Prehistoric people gathered and ate foods from a huge range of plant species, but once they began domesticating, it was *annual* plants that they transformed. Among the staple crops in Table 1.1 that yield edible reproductive biomass, the banana is the lone herbaceous perennial. Herbaceous, *grain*-producing, perennial species are not to be found at all among the world's crops plants (Cox *et al*., 2002). Herbaceous perennials generally produce less seed in a season than do annuals. Also, rapid climatic change across the Asian continent at the end of the Pleistocene dramatically increased the availability of those annual, seed-producing species that attracted the attention of cultivators (Whyte, 1977). The difference in seed production between annuals and perennials is a result of contrasting selection pressures during the two groups' evolutionary histories. Selection pressure applied in yet a different direction by plant breeders can increase seed yield and produce perennial grain crops (DeHaan, Van Tassel and Cox, 2005), but only if the right combination of breeding objectives is established.

When we think of how many civilizations built on annual cropping have fallen not to the sword but to the plough (Hillel, 1991; Lowdermilk, 1953) and the soil degradation that continues to haunt agriculture today, we can only lament the fact that the domesticators did not focus more on erosion-resistant perennial species. Apparently, ancient gatherers did utilize the seed of perennial species as food. Weiss *et al.* (2004) identified charred seeds from 3 perennial and 12 annual species of small-grained grasses that people were consuming 23 000 years ago at a site in what is now Israel. Bohrer (1972) discussed traditional methods of harvesting seed from assorted perennial grasses in Poland, Mongolia and North America. Harlan (1989a) listed a wide range of perennial grasses that people living south of the Sahara have harvested for food. Perennial lymegrass (*Leymus arenarius*) was probably cultivated by Vikings before barley reached Scandinavia (Griffin and Rowlett, 1981). Yet no domesticated perennial grain species were handed down to us by the first plant breeders.

Perennials did not compete well with annuals in disturbed soil and would not have followed people back to the fertile, churned soil around their dwellings; if some plants did happen to make their way there, they would have been overwhelmed by repeated disturbance and competition from weedy annuals. More importantly for Neolithic domesticators, farming and plant breeding were one and the same activity. As a result, they inevitably carried plant populations rapidly through sexual cycles, thereby fulfilling an essential requirement of gene-frequency change. Perennial plants re-growing

from vegetative structures would have been much more vigorous than either volunteer seedlings or intentionally sown plants; therefore, even if people tried to cultivate perennials, they would have felt little incentive to sow new generations from seed.

As we shall see, the act of sowing harvested seed applied strong selection pressure. Selection for non-shattering was strengthened when people began tilling new land year after year to sow their seed, perhaps as a part of shifting cultivation to avoid build-up of non-domesticated weeds (Hillman and Davies, 1990). Stands of perennial plants on undisturbed land would have been much less vulnerable to weeds, much more poorly adapted to shifting cultivation, and therefore less susceptible to domestication. One harvest method that spurred selection for seed retention in the annual cereals—uprooting of the plant (Bohrer, 1972; Hillman and Davies, 1990)—is very difficult with most perennials.

Woody perennials of the Mediterranean and west Asia—including olive (*Olea europaea*), grape (*Vitis vinifera*), fig (*Ficus carica*) and date (*Phoenix dactylifera*)—were domesticated in the same region as cereals, but by descendants of the first plant breeders, several millennia after agriculture had been well established (Zohary and Spiegel-Roy, 1975). Fruit-producing trees and vines did not have to compete with annual counterparts for humans' attention. They were vegetatively propagated, and, even today, most sexual progeny derived from them are "not only economically worthless, but often regress towards the mean found in spontaneous populations, showing striking resemblance to the wild form" (Zohary, 1984). The lack of far-reaching genetic changes in Mediterranean tree crops is also manifested in their failure to spread very far beyond their original climatic range, in contrast to annual domesticates from that region (Zohary and Spiegel-Roy, 1975).

Of course, early farmers also practised selection in vegetatively propagated herbaceous species. As with woody species, they selected clones with desirable characteristics – often the results of unusual mutations – and distributed them far and wide. Occasional hybridization or somatic mutation fuelled some continuing selection; for example, spontaneous yam (*Dioscorea* spp.) clones selected for cultivation by present-day farmers in Benin either are wild or are hybrids between cultivars and wild yams (Scarcelli *et al.*, 2006; Mignouna and Dansi, 2003). But with only rare sexual recombination, there was little opportunity for the degree of domestication seen in grain crops (Zohary, 2004).

The earliest plant breeders' disproportionate attention to seed-propagated annual plants has been replicated by most modern students of plant domestication. That preference will be evident in the range of examples on which the following sections draw.

1.3 INITIAL SELECTION WITHIN SPECIES

It is widely recognized that crops were not domesticated simply through gathering or cultivation. Even the most intensive harvesting of cereals does not apply sufficient selection pressure to domesticate a crop fully. Intentional sowing, in contrast, applies strong, unconscious selection pressure (Zohary, 2004). Alleles for non-shattering, lack of dormancy, reproductive determinacy and increased fertility of formerly sterile florets are all favoured by the sowing-harvesting-sowing cycle (Harlan, De Wet and Price, 1973).

In the west Asia of 10 000 years ago, wild cereals grew naturally in large fields of near-monoculture, but they were not a food source that could simply be browsed

at one's convenience. The time between full ripening and total loss of seed through shattering was only a week or two, and with hot dry weather, the period was shortened to two or three days (Zohary, 1969). Gatherers would have needed to be as timely in their harvest as today's farmers, but the harvest season was lengthened somewhat by differences in time of maturity among different cereal species and by elevation differences in the hilly Levant. Staggered harvests would have allowed people to amass large quantities of grain with a relatively long shelf-life. At the heart of the wild cereals' native range, people could obtain reliable harvests from naturally re-seeded stands; it is therefore most likely that the west Asian grain crops were first domesticated at the fringes of their progenitors' distributions (Harlan and Zohary, 1966). It was there that people would have found intentional sowing most helpful in maintaining stands of their proto-crops. At the same time, Willcox (2005) emphasized the patchiness of wild wheat stands throughout the area where emmer wheat was domesticated. People may have felt some incentive to sow seed, thereby initiating domestication, in any productive localities in that area where wild wheat was not already growing.

A study by Hillman and Davies (1990) deserves to be discussed at some length, because it takes into account many of the factors that affect methods and rates of domestication in grain crops. They started by calculating that the rare, recessive mutations for non-shattering that were necessary for domestication of the west Asian cereals were likely to have appeared once every 5 to 20 years in a typical-sized plot tended by an early cultivator. In predominantly self-pollinating wheat and barley, plants homozygous for recessive non-shattering alleles would have appeared the following season. At that point, they write, "farmers gathering their first seed stocks from wild stands will have been totally unaware of the existence of these tough-rachised mutant forms, and they would have remained oblivious of them as long as the crop stayed in its essentially wild state."

Beating spikes or panicles into a basket is the most time-efficient way to harvest wild grain crops (Hillman and Davies, 1990), but it does not apply selection pressure for non-shattering. Harlan (1967) famously collected wild cereals at the rate of 1 kg/hr by hand-stripping of spikes, but that method would not select effectively against shattering either (Hillman and Davies, 1990). Sickling or uprooting ripe or partially ripe crops does apply selection pressure, because it shakes loose some seed from wild-type plants, seed that is lost to the harvester. Hillman and Davies (1990) found experimentally that a consistently low 40 percent of wild-type seed was recovered by sickling or uprooting. Under those conditions, selection would strongly favour genes for non-shattering.

In their simulations, such strong selection intensity, combined with the high degree of self-pollination typical of wheat and barley, would have resulted in complete fixation of a recessive non-shattering gene within 20 to 30 harvest seasons, if people sowed seed each year on 'virgin land'. They further predicted that even if early farmers inadvertently relaxed the selection pressure by harvesting less fully ripened plants or repeatedly sowing on the same land, domestication would have been completed within two to four centuries. It is no wonder that we know so little about the mechanics of domestication, according to Hillman and Davies (1990). If it came and went as quickly as they envisioned it, the process was "unlikely to be preserved on

most Mesolithic or Neolithic [archaeological] sites as a recognizable progression".

Having assumed in their analysis that initial domestication was entirely unconscious, Hillman and Davies (1990) then demonstrated that even if Neolithic farmers had practised intentional selection, they could not have greatly speeded up the process. With conscious selection, people could have done no better than halve the length of time required for domestication, because they could have started selecting only when the mutants were frequent enough to be obvious, perhaps at a frequency of 1 to 5 percent of the stand. By that point, the frequency of mutants had already passed through a lag phase and was poised for a rapid increase in frequency, even under unconscious selection.

What if, because of a thunderstorm or perhaps an excessive delay in harvest, the only intact spikes from which new seed stocks could be recovered were those of mutants? Could domestication have occurred in a single season? Hillman and Davies (1990) discounted this possibility, based on variation in ripening time and the likelihood that birds or other animals would find the isolated spikes before humans did. Nevertheless, any environmental factor that hastened shattering could have increased the selection pressure and speeded up domestication.

Hillman and Davies's argument begs the question of why early cultivators resorted to sickling or uprooting, if beating is the most time-efficient harvest method for wild cereals. They suggested three reasons that sickling or uprooting apparently was preferred at some point: (1) it recovered more seed per unit land area (which, as people became more settled, may have become a more important criterion than seed quantity per unit time); (2) it permitted utilization of the straw for fire-lighting and brick-making; and (3) it may simply have become customary during a series of wet summers when wild cereals did not shatter as readily and the beating method of harvest was inadequate.

When wild cereals of west Asia shatter, their morphologically distinct basal spikelet remains attached to the culm. That spikelet would have been recovered by harvesters who sickled or uprooted plants, but not by those who gathered already-shattered spikelets from the ground. Basal spikelets might also have been left behind by hand-stripping, but that technique requires that grain be harvested before it is fully ripe, to avoid loss through shattering. Among wild barley and wild emmer remains from four archaeological sites greater than 11 000 years old, Kislev, Weiss and Hartmann (2004) found no basal spikelets and a miniscule number of unripe grains. These observations, they maintained, point to ground collecting as the original harvest method among pre-agricultural people of the region. The authors experimented with ground collection, finding that at any time during the region's rainless summer they could pick up large clumps of spikelets by grasping the upward-pointing awns.

Kislev, Weiss and Hatmann (2004) reasoned that after the first autumn rains, ground gatherers would have noticed seedlings sprouting from spikelets, and that sight would have inspired them to sow a portion of their harvested seed. Of course, sowing of ground-collected seed would have selected not *against* but *for* shattering. Kislev, Weiss and Hatmann (2004) do not speculate on how the transition to sowing of non-shattered seed occurred, but a scenario based on their results comes to mind. In collecting seed from the ground, people would have been moving slowly through stands of wild cereals long after full ripening. Any tough-rachised mutant

with its spike still intact atop the culm may have attracted their interest, and they may well have collected it for sowing in a special plot; if that happened, it would have been a very early case of intentional breeding.

Using lentil (*Lens culinaris*) as a model, Ladizinsky (1987, 1993) showed how domestication of west Asian legumes might have followed a sequence different from that of cereals. He noted that wild lentil (*L. orientalis*) plants are tiny, requiring that an estimated 10 000 plants be gathered in order to obtain one kilogram of clean grain. Therefore, lentils could not have been a major part of the gatherers' diet, as were cereals, which could be gathered much more quickly (Harlan, 1967). Furthermore, Ladizinsky argued, there would have been no incentive for sowing; an incipient lentil farmer would have had to sow their entire harvest simply to produce another crop of equal size. That is because each wild lentil plant produces only about ten seeds, of which only one seed on average will germinate the first year, given the seeds' strong dormancy.

Lentils and perhaps other pulses differed from cereals, argued Ladizinsky (1987, 1993), in that at least partial domestication had to *precede* sowing. Through intensive harvesting, people would have drastically curtailed natural reseeding, thereby leaving fields more open to fast-germinating mutants and selecting against seed dormancy. Once dormancy was largely eliminated and people were able to sow seed to good effect, selection pressure for indehiscent, non-shattering pods would have been feasible. But traditional harvesters in southwest Asia uproot lentil plants before full maturity, then sun-dry and thresh them—a process that largely avoids shattering. If that was the harvest method in Neolithic times, selection for non-shattering would have been much weaker in legumes than in cereals.

Zohary (1989) forcefully rejected Ladizinsky's model, arguing that legume and cereal domestication followed very similar paths, starting with cultivation and sowing of the wild progenitors. He maintained that wild lentils can produce not ten, but rather 40 to 70 seeds per plant when well tended in fertile soil; therefore, people might well have found sowing to be worthwhile. Ladizinsky (1989a) responded that the fields of early, inexperienced cultivators would not have been very conducive to high yields, and that conditions would have been more like those encountered by wild legume stands than those in Zohary's (1989) tilled, weeded and well fertilized experiments.

Some researchers have concluded that domestication was a rapid process in the crops they have studied, certainly when compared with evolution through natural selection. Harter *et al.* (2004) estimated that in sunflower, "genetic composition of the domesticates has changed at least 50-fold faster than the wild populations since they diverged." Wang *et al.* (1999) calculated that it took approximately 300 to 1 000 years to completely fix the crucial domestication gene *tb1* that telescopes the lateral branches in maize. Other studies indicate a somewhat slower process. Jaenicke-Despres *et al.* (2003) found that as far back as 4 400 years ago, modern mutant alleles of the genes *tb1*, *pbf* (prolamin box binding factor) and *su1* (starch debranching, which affects tortilla quality) were common. But that was almost 2 000 years after the date of the oldest known archaeological evidence of maize domestication. Based on archaeological evidence from northern Syrian Arab Republic and southeastern Turkey, Tanno and Willcox (2006) argued that "wild cereals could have been cultivated for over 10 000 years before the emergence of domestic varieties", partly because Neolithic cultiva-

tors may have taken care to harvest grain before any of it began shattering. That would have reduced the selection pressure on alleles for non-shattering. Fuller (2007) argued that during the domestication of rice, einkorn and barley, selection for grain size proceeded faster than selection for non-shattering, but that grain-size increases were much slower in pearl millet and leguminous crops. Surveying the archaeological data, he found significant grain-size increases in Asian cereals within a matter of centuries, a result, he reasoned, of the advantage large seeds had when early cultivators sowed them deeply in tilled soil. In contrast, he concluded, shattering was not fully eliminated for 1 000 to 2 000 years.

Gepts (2002) concluded that models based on a few genes can estimate only the minimum duration of the domestication process, whereas archaeological data provide a 'reality check'. Physical remains often indicate that domestication took much longer than would be predicted by genetic models.

Whether farmers' transformation of various wild plants into crops went quickly or slowly, it was not always permanent. False starts on the road to domestication may have been common. At sites in west Asia and North America, groups of people practised relatively intense cultivation of wild progenitors, and even partially domesticated some species before eventually abandoning them; those orphaned plant populations did not contribute to the founding gene pools of today's crops (Weiss, Kislev and Hartmann, 2006). In one dramatic example of that phenomenon, domestic rye may have arisen 10 000 years ago in the Syrian Arab Republic and Anatolia, only to disappear for several millennia before being re-domesticated in Anatolia and Europe (Willcox, 2005).

1.4 THE DOMESTICATION BOTTLENECK AND GENE FLOW

The number of domestication events experienced by individual species has long been a favourite topic of debate among researchers. Blumler (1992) and Zohary (1999) have argued that multiple domestications within a species have happened only rarely. They pointed out that genetic variation is much greater in most wild progenitors than in derived domesticates. They also noted the rarity of parallel domestication in related taxa above the species level. For example, people selected einkorn wheat (*Triticum monococcum*), pea (*Pisum sativum*: Ladizinsky, 1989b), emmer wheat, maize and chickpea from their wild ancestors while leaving sympatric, phenotypically similar, closely related species undomesticated.

Matsuoka *et al.* (2002) detected a single domestication event in maize by analysing microsatellite variation. Based on amplified fragment length polymorphism (AFLP) variation, Heun *et al.* (1997) concluded that einkorn was domesticated only once, in southeastern Turkey, but that result has been challenged on archaeological and climatic grounds (Hole, 1998; Jones, Allaby and Brown, 1998). Willcox (2005) summarized archaeological evidence indicating that einkorn, emmer and barley all experienced multiple domestications.

Noting that evidence for single versus multiple domestication events in Andean crops such as amaranth and peppers is inconclusive, Blumler (1992) cited several factors that render it "seldom if ever possible to rule out multiple independent invention": the progenitor species may have diversified after domestication of the crop; loci used in comparing the wild and cultivated types may be linked to loci affecting traits of domestication or ecological adaptation; or sampling by researchers may be unknowingly

biased. In a simulation study, Allaby and Brown (2003) showed that analyses relying on anonymous genetic markers might provide seemingly conclusive evidence that a species was domesticated through a single event when it was in fact domesticated more than once.

The people of South America and those of Mesoamerica probably took the common bean through two separate domestications (Sauer, 1993). Xu *et al.* (2002) concluded, on the basis of chloroplast DNA variation, that the soybean had a polyphyletic origin, but cluster analysis of nuclear random amplified polymorphic DNA (RAPD) markers indicated that local differentiation of soybean occurred in farmers' fields after domestication was complete (Xu and Gai, 2003). In any case, the soybean passed through a very tight domestication bottleneck (Hyten *et al.,* 2006). Barley is unusual among the west Asian cereals in harbouring a high level of genetic polymorphism. Ladizinsky (1998) concluded that early cultivators must have selected at least 100 non-shattering mutant plants in order to capture the level of variability seen in barley. Because the crop is highly self-pollinated, post-domestication gene flow from its wild progenitor *Hordeum spontaneum* cannot have accounted for the high degree of variability that is evident today (Ladizinsky and Genizi, 2001).

Whatever the initial number of domestication events, it is clear that because of genetic drift the diversity of most crop species is low compared with that of their wild ancestors. Drift results from a genetic 'bottleneck', usually at the point of initial domestication—the well known 'founder effect' (Ladizinsky, 1985). A bottleneck could also be caused by some later event, but generally would have to occur very early in the history of the crop, before people had a chance to distribute it over a large geographical area.

The founder effect often occurred when domestication depended upon rare mutants, but it was most severe when natural amphiploids (doubled interspecific hybrids) were domesticated. A rare amphiploid taken under human care, as was bread wheat, would have represented a gene pool consisting of a single plant—the tightest possible genetic bottleneck (Cox, 1998).

Tenaillon *et al*. (2004) found that loss of diversity in maize relative to teosinte was only 20 percent for putatively neutral loci, compared with 65 percent for loci affected by selection for traits of domestication. They estimated that the bottleneck that caused this mild contraction of variability had a ratio of population size to duration ranging from approximately 2 to 5. That is, the bottleneck population might have consisted of 10 000 plants over 2 000 generations, or perhaps 2 000 plants over 1 000 generations. Based on data from the *Adh-1* locus, Eyre-Walker *et al.* (1998) estimated a bottleneck size/duration ratio for maize of approximately 2; assuming that domestication took 300 years—similar to the duration estimated for einkorn wheat—they envisioned a bottleneck population of only 600 plants.

Sunflower apparently went through a 'substantial' domestication bottleneck, with inbreeding levels of Native American landraces varying from 0.3 to 0.5 (Harter *et al*., 2004). Abbo, Berger and Turner (2003) counted three successive bottlenecks that tightly restricted the genetic variability of the chickpea crop from its earliest days onward: the highly restricted distribution of its wild ancestor *Cicer reticulatum*; the founder effect resulting from domestication; and an early shift by west Asian farmers from autumn to spring sowing of chick-

pea (to avoid crop loss due to the *Ascochyta* blight disease). That shift required selection of plants without a vernalization requirement. This third bottleneck, which, they argue, occurred early in the crop's history, affected chickpea uniquely among the major west Asian crops. However, it reminds us that many species may have passed through bottlenecks caused by intense, early farmer-directed selection for traits other than seed non-dispersal and lack of dormancy.

Haudry *et al.* (2007) found that domesticated emmer wheat showed a 70 percent loss of nucleotide diversity relative to its progenitor *Triticum dicoccoides*. Durum wheat, derived by further selection from emmer, showed an additional diversity loss, for a total loss of 84 percent. Bread wheat's diversity unexpectedly showed only a 69 percent loss relative to *T. dicoccoides*, suggesting extensive introgression from tetraploid wheats during the 8 000 years since the origin of bread wheat.

Finally, we should take note of a much more recent, possibly catastrophic, bottleneck. Clement (1999) documented 138 Amazonian plant species—the bulk of them either fruits, nuts or vegetables—that were in 'an advanced state of domestication' at the time of the first contact with Europeans five centuries ago. Because these species had become to some extent dependent on humans for their propagation, Clement maintains that the cataclysmic post-1492 loss of 90 to 95 percent of the area's human population resulted in an approximate 90 percent loss of genetic diversity in plant species then under cultivation.

Introgressive hybridization between domesticates and their wild or weedy relatives has often expanded genetic diversity, counteracting the effects of the domestication bottleneck. Hybridization among domestic, weedy and wild populations is often an important source of new variation in crops (Harlan, De Wet and Price, 1973; Small, 1984). People tend to remove from a field those weedy hybrids that do not suit their needs, and those weeds tend to be less competitive in the natural environment as well. However, when weeds managed to backcross to crop plants, their less weedy-looking progeny might well have escaped the early cultivator's hand or hoe, remaining in the domesticated population and exchanging genes with it. Weeds often migrate over larger areas than domesticates and jump from one domesticated population to another, exchanging genes along the way (Small, 1984).

Sang and Ge (2007) attempted to reconcile seemingly contradictory evidence regarding the origin of the two rice subspecies *indica* and *japonica* by showing that the current genetic situation could have arisen from either one or two initial domestications, followed by gene flow from the two potential wild progenitors or between the partially domesticated subspecies, or both. It follows, they wrote, that introgression practised by modern plant breeding programmes is, in effect, "the continuation of domestication".

Weeds unrelated to the crop have at times enticed humans to adopt and domesticate them as secondary crops. The ancestors of oats (*Avena sativa*) and rye (*Secale cereale*), for example, caught the eyes of cultivators while growing as weeds in European wheat and barley fields (Holden, 1976).

Through analysis of microsatellites, Matsuoka *et al.* (2002) determined that the genetic diversity of maize was expanded greatly by introgression from teosinte. Wilkes (1977) found maize farmers in the Nobogame Valley of Mexico encouraging the growth of teosinte near and even within their maize fields. They told Wilkes

that the teosinte germplasm makes kernels 'more flinty and stronger'. Nobogame was the only area in which Wilkes found hybridization intentionally fostered, and, interestingly, it was the only place where the flowering times of maize and teosinte were somewhat synchronized. In other areas, people weeded out teosinte, but, at least in Chalco, they fed it to cattle as fodder, then inadvertently returned its seed to the field when applying manure. It is possible that such mechanisms also played a part in the introgression of teosinte genes into maize in the early phases of domestication.

Gene flow into crops has been important in crop evolution, but there is a much larger flow in the opposite direction: from the domesticate into the wild form. That would probably have been the case in Neolithic grain fields as well. Migration of large amounts of wild pollen into fields of self-pollinated crops was limited, and because pollen from the wild conveyed dominant genes for shattering, hybrid progeny were not likely to be collected or planted by farmers (Ladizinsky, 1985). At the same time, there is much evidence that genes regularly migrated out of fields and into wild populations (Ladizinsky, 1985; Harlan, De Wet and Price, 1973). Many studies have estimated hybridization rates by looking for crop-specific alleles in populations of the crops' wild relatives growing at various distances from cultivated fields. They generally find surprisingly high rates, even hundreds of metres away (Ellstrand, 2003).

Differences among crop species in the sizes of their founding populations and subsequent opportunities for gene inflow from the wild have profoundly affected the levels of genetic diversity available to present-day plant breeders. Here, the contrast between bread wheat and grain sorghum is instructive (Cox and Wood, 1999). Hexaploid bread wheat may well have originated from only one or two hybrid plants with genomic constitution ABD (Cox, 1998; Haudry *et al*., 2007). The tetraploid ancestor (AB) had experienced only limited introgression from diploid plants, mostly of the A-genome species. Subsequent gene flow from AB into ABD wheat plants occurred to some extent (Haudry *et al*., 2007), but gene flow from the extremely diverse D-genome donor *Aegilops tauschii* into bread wheat was either non-existent or extremely rare until it was done by twentieth-century plant breeders (Cox, 1998). Therefore, throughout the entire bread wheat species, there is limited genetic variability in the A and B genomes, while its D genome contains only a tiny fraction of the diversity found in *Aegilops tauschii* (Reif *et al*., 2005).

In contrast, people of Africa have always grown grain and fodder sorghum in areas where the crop comes into close contact and interbreeds with wild sorghum races (Doggett and Majisu, 1968). They probably domesticated sorghum in various, widespread locales on multiple occasions, after which it was exposed to a continuous inflow of variability from the wild and weedy gene pools. As a result, grain sorghum today harbours vastly more genetic diversity than does bread wheat (Cox and Wood, 1999).

1.5 GENETIC CONSEQUENCES OF SELECTION

Van Raamsdonk (1995) proposed that most domesticated crops were developed through one of four genetic models (Table 1.2). The models differ in the role of ploidy and the degrees and mechanisms of reproductive isolation. Differences in genetic and

cytogenetic mechanisms meant that the key role of the domesticator varied from model to model (Table 1.2). For instance, with some crops, people functioned as matchmakers, bringing species into contact for the first time; in others, they enforced reproductive isolation.

Domestication tends to intensify the degree of inbreeding in seed-propagated species (Zohary, 2004). The inflorescences of tomato, chili and eggplant (*Solanum melogena*), among other species, were unconsciously selected by domesticators to have shorter styles, which promoted self-pollination (Rick, 1988; Pickersgill, 1969). Artificial selection can push largely self-incompatible populations toward self-compatibility (Rick, 1988), as is believed to have happened in types of *Brassica oleracea*, including summer cauliflowers (Thompson, 1976). Here, there is a kind of ratchet effect: disruption of self-incompatibility systems is easily accomplished, whereas selection in favour of self-incompatibility would have been genetically complex and very difficult (Rick, 1988).

Inbreeding is a powerful accelerator of unconscious selection for traits governed by recessive genes. The fixation of genes for non-shattering that might have required only a few centuries in highly self-pollinated wheat and barley would, with 100 percent cross-pollination, have taken more than 8 000 years (Hillman and Davies, 1990)!

Each of two recessive alleles at different loci in domesticated rice that reduce seed shattering resulted from single-nucleotide substitutions (Li, Zhou and Sang, 2006; Konishi *et al*., 2006). Five of six well studied domestication genes in maize, wheat, rice and tomato exhibit differences in regulatory regions between the wild and domestic alleles

TABLE 1.2

Four models proposed by van Raamsdonk (1995) by which the genetic mechanisms of crop domestication can be classified, along with his lists of crops that exemplify each model and some crucial points at which humans intervened in the domestication process under each model

Domestication model	Examples	Crucial actions by domesticators
Reproductive isolation between a diploid domesticate and its diploid wild ancestor is caused by internal barriers, post-zygotic barriers, external reproductive barriers or apomixis.	Soybean, common bean, chickpea, lentil, cowpea (*Vigna unguiculata*), lettuce (*Lactuca sativa*), citrus fruits (*Citrus* spp.)	Selection for self-pollination and against weedy hybrids; fostering of genetic drift
Development of crop-weed-wild complexes in which genetic information is exchanged more or less freely among diploid domesticates and their sexually compatible wild progenitors.	Maize, rice, barley, grape, sorghum, pearl millet, foxtail millet (*Setaria italica*), radish (*Raphanus sativus*), beet (*Beta* spp.), chili (*Capsicum* spp.), quinoa (*Chenopodium quinoa*)	Adoption of weeds that invade cultivated land; toleration or encouragement of weeds that can backcross to less wild cultigens
One or more rounds of hybridization and polyploidization occur among wild species prior to domestication.	Cotton, sweet potato, groundnut, tobacco (*Nicotiana* spp.), cucumber (*Cucumis* spp.), coconut (*Cocos nucifera*), alfalfa (*Medicago sativa*)	Selection at the polyploidy level
Interspecific hybridization involving at least one domesticated species is followed by polyploidization. Resultant amphiploids are reproductively isolated.	Bread wheat, potato, banana, coffee (*Coffea arabica*), yam (*Dioscorea* spp.)	Bringing formerly isolated plant populations into contact; selection and propagation of rare amphiploid plant(s) found in or near cultivated fields.
In some cases, domestication occurs through a combination of mechanisms from more than one of the above models.	Sugar cane, oat, *Brassica* spp., tomato (*Lycopersicon esculentum*)	—

(Doebley, Gaut and Smith, 2006). Whatever the nature of their mutations, alleles initially selected by domesticators often showed the simplest modes of inheritance. Many genes governing traits of domestication are recessive or additive, and would have been expressed more strongly among the progeny of plants that tended to self-pollinate most frequently. An increased tendency to inbreed may also have been an indirect result of selection for higher grain yield; self-pollination ensures seed and fruit development, especially if the new crop was transported out of the range of its natural pollinators.

Inbreeding also leads to greater within-line uniformity, but it is hard to imagine uniformity being a direct selection criterion for early domesticators, as it would have required that they plant out the progeny of individual plants in separate plots. It is almost certain that they practised mass selection, not progeny testing. But genes promoting self-pollination might have been favoured in very small populations maintained in isolation. Such isolation could have resulted from individual preferences, or perhaps community customs, such as a belief in parts of Guatemala that plants should be grown only from seed produced on the same plot of ground (Pickersgill, 1969). 'Colour coding' (Wilkes, 1989) based on endosperm pigmentation may have helped farmers maintain small, genetically isolated maize populations.

Strong selection to reinforce inbreeding did not occur in crops that were propagated vegetatively; in them, self-incompatibility and out-crossing remained common (Zohary, 2004; Rick, 1988). Through clonal propagation, cultivators could produce large, genetically desirable populations. In contrast to seed-propagated species, in which human selection for improved grain harvests also reinforced meiotic stability, selection in vegetatively propagated species allowed, or even encouraged, variations in chromosomal number and structure, disrupting reproductive development to varying extents (Zohary, 2004).

In a simulation study, Le Thierry d'Ennequin *et al*. (1999) predicted that to fix a full complement of alleles for domestication, either linkage among loci or a significant degree of reproductive isolation is essential. By their models, in predominantly self-pollinating species subject to little migration, people easily fixed alleles at unlinked loci through selection; however, in species with a high degree of out-crossing, human selection favoured blocks of linked domestication genes.

Empirical experiments have demonstrated that linkage among domestication loci is common, regardless of breeding system (Paterson, 2002). In crosses between pearl millet and its wild progenitor *Pennisetum mollissimum*, Poncet *et al*. (1998, 2000, 2002) found linkage among genes affecting spike characters—important components of the domestication syndrome—but not among genes affecting vegetative characters or total grain yield. Burke *et al*. (2002) mapped 78 quantitative trait loci (QTLs) affecting 18 traits in a cross between sunflower and its conspecific wild progenitor. The domestication-associated loci were spread across 15 of 17 linkage groups, but were highly clustered within those groups. Both pearl millet and sunflower are highly cross-pollinated. In rice, a selfing species, QTLs affecting domestication traits also tended to be clustered in linkage groups (Cai and Morishima, 2000).

Wright *et al*. (2005) found that 2 to 4 percent of the genes in maize have probably undergone artificial selection. Much of that selection, especially for the genes involved in plant growth and auxin response that are responsible for the dramatic differences in

plant morphology between teosinte and maize, appears to have occurred during initial domestication. Those growth-pattern genes were clustered, whereas genes affecting amino acid composition were not.

In wild progenitors, significant numbers of agronomically beneficial alleles are often embedded in linkage blocks with other, deleterious, genes. Such desirable alleles tended to be left behind during domestication. For example, in a tetraploid wheat population, Peng *et al*. (2003) found 24 percent of positive QTL effects to be coming from the wild *Triticum dicoccoides* parent. By breaking up such linkage blocks, modern-day breeders can utilize genes that were 'hidden' from early domesticators.

Gepts (2002), surveying studies of domestication traits in maize, pearl millet, common bean and rice, found an average of 2.2 to 5.3 loci per trait. Those loci accounted for only about 50 percent of the total variation per trait, and loci affecting all traits were spread among 3 to 5 linkage groups per species, indicating rather diffuse genetic control. Paterson (2002) found similar patterns in the QTL-mapping literature on sorghum, rice, maize and tomato. He concluded that loci with larger statistical effects were probably biologically significant as well, because they occurred in similar genomic regions in different crop species (Paterson, 2002; Paterson *et al*., 1995).

During domestication, people may have unknowingly favoured plants or populations with a higher inherent rate of recombination per unit of physical chromosomal length. A comprehensive survey showed that mean numbers of chiasmata per bivalent were significantly higher in 46 crop species than in 150 wild species (Ross-Ibarra, 2004). This result was in accord with theory, the bulk of which predicts that an increased rate of recombination is favoured during periods of rapid evolutionary change, of which domestication is an extreme example. Ross-Ibarra found no support for the alternative possibility: that species with higher recombination rates are 'pre-adapted' to domestication.

Even under domestication, the recombination rate is under stabilizing rather than unidirectional selection, because the same high rates that help break up repulsion linkages also speed up the decay of co-adapted gene complexes (Dobzhansky, 1970). Indeed, Ross-Ibarra's comparison of crop and wild species provided evidence for selection against excessive recombination. There are, of course, other mechanisms for maintaining favourable multilocus combinations, including paracentric inversions (Dobzhansky, 1970) and self-pollination (Clegg, Allard and Kahler, 1972).

1.6 INTENTIONAL SELECTION

Although crops were domesticated through largely unintentional selection, there is little doubt that the domesticators quickly became aware of their own ability to change the phenotypic composition of their crops from generation to generation. Genetic modification, once initiated, spread in ever-widening ripples through plant genomes. Sowing spurred unconscious selection for traits like non-shattering; changes caused by unconscious selection prompted observant farmers to practise intentional selection; and intentional selection for one trait often affected other traits as well, through linkage and pleiotropy. Studies of a grain-quality trait in rice show that human selection at a single locus can exert very strong selection pressure on a large chromosomal region surrounding it, causing a so-called 'selective sweep' that can affect other traits much more strongly than would natural selection (Olsen *et al*., 2006).

From the dawn of agriculture until the twentieth century, farmers acted as plant breeders, working almost exclusively through mass selection; that is, by ensuring that some individual plants made a proportionately greater genetic contribution to the following generation than did others. Natural out-crossing would have been frequent enough, even in highly self-pollinating species, to generate useful genetic recombinants. Early plant breeders worked without the benefits of progeny testing or replication, both of which can enhance gain from selection, but they had two other important factors working in their favour: time and ecosystems. Even small gene-frequency changes from year to year translated into large improvements when they continued over vast numbers of growing seasons. And plant populations upon which people exerted gradual selection in a particular locality, through the full range of weather conditions and pest, pathogen, weed and intercrop populations that the locality had to offer, were bound to be resilient and reliable food producers.

When people applied direct selection pressure for some traits, whether intentional or unconscious, they put indirect selection pressure on others. For example, attached glumes increase seed dormancy, so selection for non-dormancy may have increased the frequency of free-threshing plants. Deep sowing may have favoured larger-seeded genotypes (Fuller, 2007), which, in turn, would have had lower grain protein concentrations via dilution. Selection for greater allocation of resources to reproductive growth (higher harvest index) could have increased susceptibility to pests (Rick, 1988). Because plant parts growing from the same meristematic regions exhibit allometric growth, selection to increase the size of one organ generally affected others; for example, selection for larger spikes in the cereals produced wider leaves and thicker culms as well.

Smartt (1969) catalogued the many traits for which early domesticators applied selection pressure in species of *Phaseolus*: a reduced number of lateral branches (to avoid excessive tangling in fields where beans were meant to climb maize plants); more robust leaves and stems; larger flowers; increased photoperiod sensitivity; increased pod and seed size; greater permeability of the testa; and reduced pod dehiscence. However, in examining four cultivated species, he found that not all of those traits were affected in every species.

Chang (1976a, b) noted a similarly increased size of vegetative organs and kernels in rice, along with a more extensive root system; higher tillering capacity; synchronization of tillering; more panicle branches; a longer grain-filling period; tolerance to non-flooded conditions; and loss of pigmentation. However, increases in kernel size and harvest index associated with domestication of rice were less than those in most other cereals (Cook and Evans, 1983). In several species of chili (*Capsicum*), people rejected erect-fruited wild plants in favour of mutants with pendant fruits, which were hidden under the foliage canopy and therefore protected from bird damage (Pickersgill, 1969).

Maize is often recognized as a crop that underwent some of the most remarkable morphological changes during domestication, but, as in most crops, the most obvious transformation was in its reproductive structures. In the words of Iltis (2000),

> *Cover the ears, and it sometimes takes a specialist to tell teosinte from maize … But compare a many-rowed, 1000-grained ear of maize to a 2-rowed, 5-to-12-grained ear of teosinte – and be perplexed! How*

could such a massive, useful monster be derived from such a tiny, fragile, inedible, useless mouse?

Perhaps just as surprising is the finding that morphological differences between maize and its wild ancestor are under relatively simple genetic control (Doebley and Stec, 1993).

Maize is not the only species whose reproductive structures evolved into monstrosities under the guiding hand of early breeders. For example, pearl millet's wild ancestor has heads measuring no more than 10 cm in length, but from it, early breeders selected cultivars with heads up to 2 m long (Harlan, 1989b). In bringing about the visually dramatic domestication of the sunflower, Native Americans selected for the fusion of many smaller heads into fewer, larger ones. People worldwide selected for often dramatically larger reproductive structures in vegetable and fruit crops.

Plant breeding theory, as well as observation of crop domesticates, tells us that the first breeders had their biggest impact on traits that (i) were of the most intense interest to the people who used the plants for food; (ii) were under relatively simple genetic control; and (iii) had a relatively high heritability on a single-plant or single-propagule basis. Therefore, humans altered the appearance and food quality of the harvested product more rapidly than they did traits such as yield per unit area. Contrasting intentional selection with the unconscious selection that preceded and paralleled it, Harlan, De Wet and Price (1973) wrote:

Deliberate selection adds new dimensions to the process [of domestication]. Human selection may be more intense and absolute and is often biologically capricious or even whimsical.

They went on to list a bewildering array of food products and processing techniques, all of which were certain to reveal genetic variation in the crops upon which they were practised.

Human selection for nutritional quality of crop domesticates occurred in the context of other crops that were evolving simultaneously. The most commonly cited example is the complementarity of amino acid profiles in cereals and legumes. Selection among and within species was a matter of health, even life and death. Indeed, Wilkes (1989) declared an 'ethnobotanical rule', stating that when "crops are consumed and not sold, a reasonable level of nutritional adequacy has evolved and been maintained". Neither the single-minded selection for high grain yield per unit area nor the pursuit of high-lysine maize would have occurred to a Mesoamerican farmer of 3 000 years ago.

Plant breeding requires differential phenotypic expression. For example, people could not venture very deeply into the domestication and improvement of a species as a food source if its consumption always resulted in serious illness or death. Indeed, the process by which the sweet almond was derived from its cyanogenic ancestor is still shrouded in mystery (Ladizinsky, 1999). People could begin selecting for lower toxicity once they accomplished at least partial breakdown of toxins through cooking. Other strategies were developed farther back in the human family tree. Geophagy—consumption of clays—is practiced by at least eight primate species (Johns, 1989). People commonly eat clay along with wild potatoes (Johns, 1986) and yams (Irvine, 1952) to de-toxify them, and the practice might have provided latitude for early domesticators to distinguish among different degrees of bitterness without falling too ill too often. Once foods were rendered edible via such practices, selection for lower

toxicity might have been furthered simply through dilution, as people selected for greater root or tuber size (Johns, 1989).

In the potato, there is a remarkable coincidence between toxic thresholds and human capacity for detection. The plant's most common glycoalkaloid is toxic in concentrations above 200 ppm (Johns and Keen, 1986), and tubers with a concentration of greater than 140 ppm are considered unpleasantly bitter by North Americans (Sinden and Deahl, 1976). In contrast, the Aymara Indians of the Andes classify potatoes with concentrations above a range of 200 to 380 ppm as bitter (Johns and Keen, 1986). Because several wild and cultivated *Solanum* species are crucial sources of calories in the Andes, the Aymara and other indigenous people may have developed a taste for somewhat riskier genotypes. Selection for improved nutritional quality can also work against improvement of other traits. For example, potato populations selected for lower glycoalkaloid concentrations had lower resistance to potato leafhopper (Sanford *et al*., 1992).

In a seeming paradox, cyanogenesis (the production of poisonous hydrocyanic acid) is more common in crop plants than in the plant kingdom as a whole. Jones (1998) noted that 16 of the world's 24 leading crop species (by total production) are cyanogenic in some plant part(s) at some stage of growth. Cyanogenesis, Jones observed, is an important mechanism of resistance to pests. People looking to become cultivators, given a wide range of plant species from which to choose, would probably have been attracted to plants that had not already been damaged or largely consumed by other species. Having the unique ability to eliminate cyanogenic glycosides by grinding, steeping and cooking, humans took advantage of plants that could not be consumed by rival species. Reducing the mean toxicity to a safer level allowed them to detect and exploit genetic variation within species.

Toxins aside, the simplification of diet that followed the expansion of agriculture appears in itself to have caused a decline in overall human health (Kates, 1994). Gepts (2002) even implies that had regulatory agencies existed in Neolithic times, domesticated plants might well have failed to receive approval!

Selection for food quality involved more than nutritional considerations. Where muscle and fuel power were resources not to be squandered, genotypes that produced food with lower energy requirements for processing and cooking may have been more highly valued. For example, Harlan (1989b) described how modern cultivators in Mali select sorghum heads with softer grains for ease of pounding, but also keep hard-seeded, more insect-resistant types, for longer-term storage.

In some cases, people may have utilized the progenitor of a crop for one purpose only to find, once they became more familiar with the species, that it possessed one or more other traits that warranted its full domestication. For example, many East Asian plants may have been used for medicinal purposes before being domesticated for food production (Chang, 1970). Bohrer (1972) maintained that the wild grasses that eventually gave rise to cereal crops were originally cut or uprooted for use as animal fodder. However, Hillman and Davies (1990) disputed that idea, arguing that at the time and place of west Asian crop domestication there were no domestic cattle and few domestic sheep or goats. The squash (*Cucurbita pepo*) may have been domesticated first for its seed, or for its hard gourds to be used as containers; once

fleshy vegetable genotypes were selected, people may have stopped growing the gourd types to prevent the appearance of bitter squashes through cross-pollination (Heiser, 1989).

Iltis (2000) concluded that teosinte was first grown by Mesoamericans for its green shoots and sugary pith and not for its grain, which remained enclosed in a hard fruitcase. Later, through increased contact with teosinte as a snack or vegetable, an alert cultivator may have noticed an extremely rare, 'grain-liberating' mutant—on possibly a single occasion—thus kicking off the process of maize domestication.

Amplifying Iltis's hypothesis, Smalley and Blake (2003) suggested and then defended a possible sequence of events by which teosinte domestication proceeded: (1) people began casually harvesting and chewing the sweet stalks and shoots of *Zea* plants; (2) they found that they could extract more juice by mechanical mashing; (3) to preserve the juice, they adopted fermentation techniques already in use with other species; (4) they spread maize far and wide, as a new resource for making alcoholic beverages; and, finally, (5) to expand *Zea* cultivation, they began sowing harvested seed. Once that sequence proceeded as far as step (5)—along with the discovery of the free-kernel mutant—domestication of *Zea mays* as a grain crop would have followed quickly. But the time between its very first utilization by chewing and its full domestication as a grain may have been as long as 2 500 years (Smalley and Blake, 2003).

Perhaps too often, researchers tend to portray the era of crop domestication as one of constant struggle against scarcity and hardship. DeBoer (2003) commented that the possibility of people first having utilized maize for sweet and fermented products.

> *...injects desirous human agents into the account, a palliative for the stern 'food crises' and 'population pressures' that haunt our angst-driven prehistories. How charming it would be to have a snack-and-party crowd, hassled by only an occasional aggrandizer or two, at the base of the Neolithic!*

The initial domestication of crops prompted expansion of farming into new environments, where people continued selection under different conditions, while perhaps repeating the domestication process with new species. Although the ability to accumulate a large excess of grain during a brief harvest season provided, in itself, a strong incentive to settle in one locality for at least a good part of the year [as Flannery (1969) asked regarding a hypothetical community of Neolithic gatherers, "…after all, where could they go with an estimated metric tonne of clean wheat?"], people eventually and inevitably migrated. The ability to take with them a food source that doubled as the means of sowing future crops allowed people to expand agriculture into previously unsettled areas, where the crops encountered new selection pressures and the people encountered new species of plants.

Abandoned fields created by early shifting cultivation in tropical forests may have provided environments in which useful wild plants could survive and grow unusually well, possibly to become domesticates themselves (Piperno, 1989). Barley's early maturity allowed farming at very high altitudes; pearl millet's drought-hardiness extended agriculture into parts of India and Africa that receive 200 mm or less of annual rainfall; and maize brought more people into the sparsely populated, mid-altitude hill country of India and Pakistan (Harlan, 1972). However, once settled in new environments, thanks to a reliable

staple crop, people have not always sought out additional species for domestication; rather, monocultures are common on the fringes of agriculture (Harlan, 1972).

1.7 CONCLUSIONS

In recent decades, institutional plant breeders have come to realize the importance of integrating breeding methodology with farmers' knowledge. Doing so has benefits for breeders—whose selection goals become more embedded in the 'real world'—and for farmers, who come to appreciate better their own ability to change gene frequencies of their crops in favourable directions. This would appear to bring us full circle, to a time like that of agriculture's earliest days, when breeding and farming were fully integrated. But today's agriculturalists also have ten millennia worth of hard-won farming and breeding knowledge on which they can draw by working together.

The first plant breeders lived in prehistoric times, so they left us no direct accounts of the methods they used to domesticate and improve crops. As we have seen, many of our hypotheses about their activities are influenced by our knowledge of the methodologies that farmer-breeders have used in historic times. That is no accident. By extrapolating recent methods back to the origin of agriculture, we are acknowledging a 10 000-year-long, unbroken thread of skills and knowledge that is derived from growing plants for food while simultaneously breeding them for the future. Nevertheless, we should not forget that by coming to rely largely on domesticated plants and animals, we humans have also lost vast amounts of knowledge of other species and ecosystems; there is much that we could re-learn from hunter-gatherer societies of the present, the recent past and the days before agriculture.

Keen observation and use of genetic variation in plant species has been a hallmark of societies that depend directly on those plants, whether the people in those societies were hunter-gatherers, the originators of agriculture, or today's subsistence farmers. As the millennia have passed, knowledge has expanded and methods have evolved, but that thread remains intact. Today's institutional plant breeders also benefit from that accumulated knowledge. Although modern breeders' methodologies are often very different, they are rooted firmly in the past. They also utilize that major part of the first plant breeders' unwritten knowledge that survives in code, the genetic code of the plants themselves.

Had the original crop domesticators been familiar with the principles of genetics, the crop species that they handed down to history might have been even more profoundly transformed. Had they understood the hazards of genetic erosion or pest and pathogen epidemics, they might have domesticated a wider range of species and avoided the genetic bottlenecks that restricted variation in many crops from the very beginning. And could they have foreseen the devastating consequences of soil erosion and water contamination under long-term annual cropping (Cox *et al*., 2006), they might have mounted an effort to domesticate resource-efficient perennial food crops.

Nevertheless, that relative handful of people was responsible for the most important turning point humanity has yet experienced, laying the foundation for the material and cultural world that surrounds us today. But in the evolution of agriculture, it has not been the case that superior knowledge and techniques continuously replace inferior ones. Knowledge survives from every era, all the way back to the origin of crops (and even well before), so that farmers, plant breeders

and all others who work in agriculture can draw upon it in the years ahead.

As it has turned out, the first plant breeders brought about changes in our own species that equal any they achieved with plants, and the plant breeding traditions they established have brought humanity, only in the past century, to a point at which we can study why and how they carried off their revolution, and learn from the answers.

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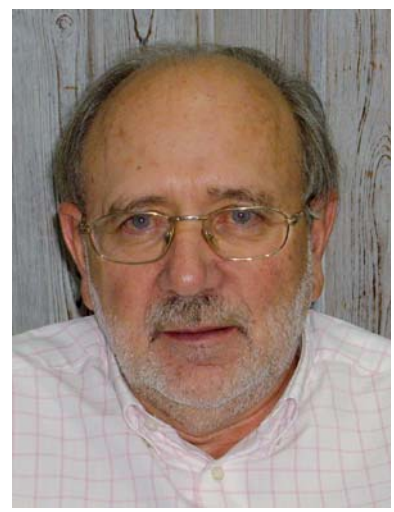
CHAPTER 2

Theory and application of plant breeding for quantitative traits

Javier Betrán, Jesú Moreno-González and Ignacio Romagosa



2.1 A HISTORICAL PERSPECTIVE

A series of accounts from Roman to modern agricultural developments are sketched to show that foundations of current plant breeding, with the significant exception of hybrid breeding, lay in pre-Mendelian times.

2.1.1 Pre-Mendelian plant breeding

Plant breeding traces back to the origin of agriculture (Harlan, 1975; Cox, Chapter 1 this book). Plant domestication through manipulation of few genes with major phenotypic effect generated most food crops early in the evolution of human civilizations. There are approximately 250 000 plant species, of which 50 000 are edible and 5 000 have economic interest, but only 250 are food crops (Sánchez-Monge, 2002). In fact, 90 percent of the calories in the human diet come from just 15 crops, and 60 percent from just wheat, rice and maize. Only a few crops have their origin in the last few centuries and, thus, most major Mediterranean crops are already listed in the Bible. Sugarbeet, celery and rubber became crops in the nineteenth century. Macadamia nut, kiwifruit, blueberries, cranberries, lingonberries and jojoba are examples of the very few post-Mendelian domesticated crops (Ladizinsky, 1998).

Alonso de Herrera published in 1513 his *Agricultura General*, the first modern treatise on agriculture. This text suggests that many current breeding methods for autogamous crops were unknowingly being developed at the turn of our era. Referring to the Roman agronomists, Virgilius, Varro, Plinius and Columella in particular, Alonso de Herrera gave general recommendations about the seeds to be used for sowing cereals. For example, similarly to current bulk selection, he recommended taking the grains from the bottom of the pile upon threshing as they were better because of their heavier weight. As we would currently do to select a genotype within a heterogeneous population using individual or pedigree selection, he suggested that

> *… whenever a plant was found with many large spikes, it should be harvested separately; its seed increased isolated from the rest until a large amount of seed could be used for further growing.*

Johannsen (1903, see Section 2.1.2 below) is considered to be the first to postulate the central plant breeding equation of *Phenotype = Genotype + Environment* (as we will see later, the equation is a little more complex). However, Alonso de Herrera more than 500 years ago stated that "no good crops are to be expected from poor seed unless favoured by good growing conditions". He also recognized the importance of specific adaptation when he wrote that the seed had to be harvested from similar conditions to those where seed was to be grown "from hot to hot, from cold to cold, from dry to dry, from mild to mild, from humid to humid, …".

Plant and animal breeding continued their extraordinary advances in the few centuries before Mendel's work. Spontaneous mutation, hybridization, introgression and crop diffusion played a key role in increasing genetic diversity of crops (Ladizinsky, 1998). Interspecific crosses were first carried out in the eighteenth century. For example, Duchesnes was the first to identify the parentage of the natural hybrid of a new strawberry now named *Fragaria* × *ananassa,* which through continuous breeding became the current big-fruited crop (Darrow, 1966).

Commercial breeding has existed for centuries. Tulip trade in the Netherlands since the beginning of the seventeenth century involved very large amounts of

money (Doorenbos, 1954). The German city of Quedlinburg became known from the middle of the nineteenth century with the establishment of profitable seed companies breeding vegetables and flowers. The Vilmorin Company, a commercial label still in operation, was established in 1743 in France, when Philippe-Victoire de Vilmorin, a horticulturist and Pierre d'Andrieux, a seed collector and botanist of Louis XV, set up the boutique 'Andrieux and Vilmorin' in Paris (www.vilmorin-clause.com). One of the members of the family, Louis de Vilmorin, a contemporary of Mendel, introduced two key techniques in modern plant breeding: first, progeny testing, as a alternative way to assess the value of a given individual based on the phenotype of its offspring rather than just its own; and, second, indirect selection, whereby sucrose yield in the beet root was measured by means of a refractometer. Continuous breeding boosted sugar content from 5–6 percent to 20 percent in just a few decades.

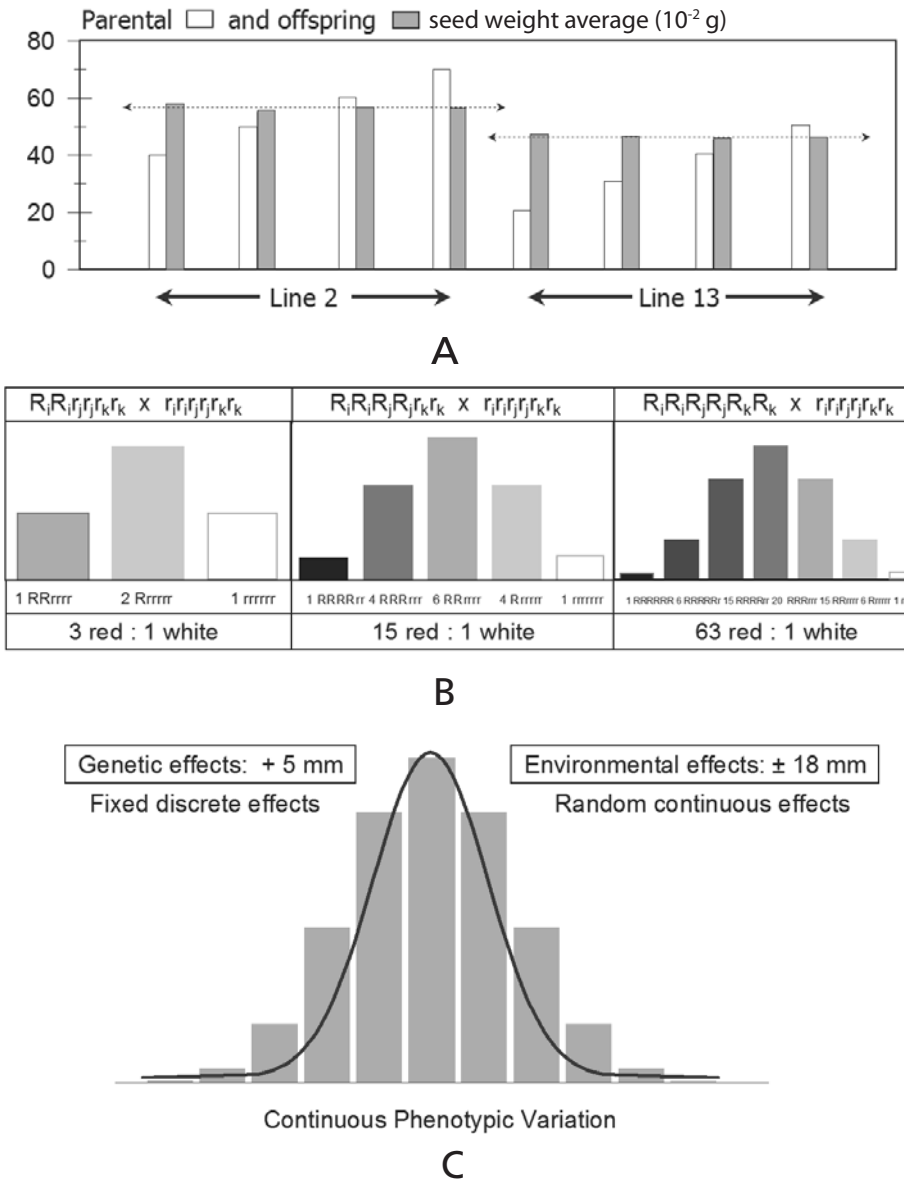
Parallel to plant breeding, by the end of the eighteenth century, animal breeding had developed intensively, based on practical experience. The great success worldwide of the breeders of the Spanish Merino sheep by the leading pioneers in this field such as Robert Bakewell (1725–1795) proved that breeding was an empirical endeavour in which ideas arising out of observation were ahead of academic knowledge. Wood and Orel (2001) summarized the ten intuitive principles and practices used at that time, most of them inadvertently compatible with Mendelian inheritance: (1) The intrinsic nature of an animal (its breed and blood) was the most critical factor determining its form and quality; (2) Good husbandry (mainly diet and housing) were essential for maximizing their intrinsic quality; (3) Transportation of animals from one country to another could be worthwhile, provided that the introduced stock was carefully bred in every subsequent generation; (4) Selective breeding was a powerful agent of change, even for creating new breeds; (5) The more inbred a strain was, the more likely to pass the selected traits to its progeny; (6) Both sexes contributed to heredity, and either could be prepotent, and thus characteristics could be transferred to the opposite sex; (7) Carefully controlled progeny testing was the most efficient way to evaluate an individual's hereditary properties; (8) Selective breeding was applied to single traits or groups of traits; (9) Visible traits could indicate hidden properties; and (10) The value of crossing, as an adjunct to selection, was still a matter of controversy, although the first generation of a cross was becoming accepted for its hybrid vigour.

2.1.2 The onset of Quantitative Genetics: the Mendelians vs. Biometricians debate, and the Neo-Darwinian synthesis

The theory of plant breeding rests on the work of two most influential biologists, Charles Darwin (1809–1882) and Gregor Mendel (1822–1884), and the passionate debate that took place between their followers at the beginning of the twentieth century.

Darwin published in 1859 *The Origin of Species*, in which he persuasively demonstrated that evolution had occurred. He then elaborated the 'Natural Selection' hypothesis to explain the evolutionary process. He backed his observations in nature with the gains of artificial selection achieved in both animal and plant breeding. He strongly supported gradual changes acting over time, rather than discontinuous or abrupt changes. However, he lacked a con-

FIGURE 2.1
Key experimental evidence in the Mendelians vs. Biometricians debate



(A) Johannsen's Pure-Line Theory (1903): heritable (selection between lines) and non-heritable (within a given line) variation for seed weight in beans. (B) Nilsson-Ehle´s Multiple Factor Hypothesis (1909): F_2 segregation ratios for wheat cultivars differing in two alleles at one, two and three loci controlling seed colour. (C) East's experiment on the corolla length in *Nicotiana longiflora* (1916): the apparent continuous variation in the F_2 could be modelled by the superimposition of environmental effects (estimated to be approx. ±18 mm) and genetic effects determined by a reduced number of independent genes (approx. ±5 mm per allelic substitution considering five loci with two alleles at each locus).

vincing theory for the origin of variation and a proper theory of inheritance. Darwin assumed the prevailing model of the blending inherence as observed in progenies of interbred animal crosses by which the attributes of an individual were the result of merging or blending their parent's characteristics (a complete fusion of parental and maternal particles called 'gemmules'). Jenkin (cited by Griffing, 1994) soon realized that under blending inheritance, the variation in the offspring would be halved after each generation of random mating and, thus, variation within any population would be quickly exhausted: if

$$x_o = \frac{x_m + x_f}{2} \quad \text{then}$$

$$\sigma^2(x_o) = \sigma^2\left(\frac{x_m + x_f}{2}\right) = \frac{1}{4}\left(\sigma^2(x_m) + \sigma^2(x_f)\right) = \frac{1}{2}\sigma^2(x_p)$$

where x_o, x_m,, x_f,, and x_p, represent the offspring, male, female and parental values, respectively. New variability should have to be generated to maintain the level of variation within the population.

Mendel's laws of segregation clearly established that any trait is determined by a pair of factors, gametes containing just one of the two factors taken at random. The law of independent assortment establishes that factors from the parents independently combine in the offspring. Mendel supplemented Darwin's Natural Selection because a direct consequence of his laws was that genetic variation could be preserved through time. Whereas genes according to Mendel were conserved over the generations, 'gemmules' received from any parent according to the blending theory were physically lost by being merged together.

Hugo De Vries, contrary to Darwinism, proposed the *mutations theory* for describing the sudden production of new species. This theory was fervently accepted by the Mendelians of the early twentieth century, led by De Vries and William Bateson. They considered the selective value of small variations negligible and believed genetic variation could not be explained statistically. On the contrary, biometricians such as Francis Galton's disciples, guided by Karl Pearson and Raphael Weldon, developed statistical techniques for describing and analysing relationships between relatives with respect to continuous variation. They strongly supported Darwinism and rejected Mendelism as just a series of simple naive general principles. As Griffing (1994) mentions, "a profound controversy developed between the two groups, augmented by the personalities of the scientists involved".

The core of the debate was whether continuous variation observed for metric characters could be reconciled with the discrete Mendelian factors and their inheritance. Mendel's laws and biometrical methods were recognized as complementary after a series of key plant experiments carried out in the first decades of the twentieth century in what Griffing (1994) wisely called the *Era of demystification and reconciliation.* Two important questions were answered: first, What are the basic causes of continuous variation? and, second, What is the nature of the genotypic variation?

Johannsen (1903) addressed the first question with his so-called *Pure Line Theory*. He studied the seed size in a heterogeneous bean variety, 'Princess', a self-pollinated species that was a mixture of pure lines. He proved that continuous selection within a pure-line did not translate into larger grains, but genetic advances could be achieved upon individual selection within a mixture of lines (Figure 2.1A). He

distinguished heritable from non-heritable variability and proposed the concept of *Genotype* and *Phenotype,* which led to the formulation of the central equation in breeding: *Phenotype = Genotype + Environment*.

The Multiple Factor Hypothesis was demonstrated by Nilson-Ehle in Sweden in 1909, answering Griffing's second question. Crossing different wheat varieties of red and white seed colour, he found distinct intensities of red to white kernel 3:1, 15:1 and 63:1 segregation ratios in the F_2 (Figure 2.1B), depending on the red variety used. He postulated that two alleles at each of three independent loci controlled the trait which increasingly showed a continuous phenotypic distribution. East (1916) published the final experiment that clearly brought together Mendelians and Biometricians. He studied the length of the corolla of progenies of two different strains of *Nicotiana longiflora,* which seemed to display blending inheritance. The apparent continuous variation in the F_2 could be explained by the superimposition of environmental effects (estimated to be approx. ±18 mm) on the corolla length determined by a reduced number of independent genes (approx. ±5 mm per allelic substitution considering five loci with two alleles at each locus) (Figure 2.1C).

The crucial work for the development of the Theory of Plant Breeding was published by Fisher in 1918. He introduced the term 'variance' and used its additive properties to partition the phenotypic variance into its components according to a genetic model. He provided the theoretical framework for the final settlement between Biometricians and Mendelians and established the basis of quantitative genetic theory, on which both animal and plant breeding rest. As Griffing says (1994),

> *… the era of the 1920s was blessed by having three of the right scientists (Fisher, Haldane and Wright) with special interest and abilities in the right area (mathematical biology) at the right time.*

They adopted Mendelian inheritance to describe in mathematical terms the basic principles of Darwinian evolution (and, thus, both natural and artificial selection). Their developments constitute the core of the plant breeding theory described below.

As a key component of genetic inheritance, Morgan, Sturtevant, Bridges and Muller clearly demonstrated, working with *Drosophila* during the 1920s, the linear order of the genes in the chromosomes. By 1926, once Morgan's book, *The Theory of the Gene*, was published, it was generally recognized that genetic maps indicating the position and order could be theoretically constructed for any organism (Stebbins, 1994). It has not been until recently, with the advent of molecular markers, that this asseveration has been fully developed. It is also worthwhile mentioning that the concepts of marker-assisted selection (MAS) and quantitative trait loci (QTL) can also be traced to original studies in beans by Sax in 1923 or in tomato by Lindstrom (1926).

Through the use of mathematical models, quantitative genetics studies the genetic architecture and heritability of plant traits, the genetic relationship among them, and the interaction between genotypes and environments. Quantitative genetics thus provides the foundation for the design and utilization of breeding approaches to improve crops. In the next section, we describe the nature of plant traits and genetic phenomena affecting their expression, ways to estimate heritability and subsequent response to selection.

2.2 THE BASIS OF QUANTITATIVE GENETICS

The theory of quantitative genetics has been developed since the early twentieth century to explain the performance, variability and inheritance of quantitative (complex) traits, which are frequently exhibited in living organisms (Fisher, 1918; Wright, 1921; Haldane, 1924; Comstock, Robinson and Harvey, 1949; Kempthorne, 1969; Crow and Kimura, 1970; Falconer, 1981; Dudley, 1982, 1984; Lande and Thompson, 1990). Complex (quantitative) traits behave as non-Mendelian factors and are assumed to be governed by several loci, each with two or several alleles and variable effects.

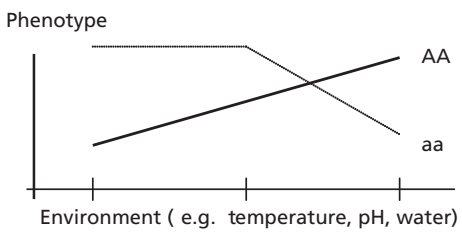
2.2.1 Quantitative traits

Quantitative traits are controlled by many genes, in contrast to qualitative traits that are regulated by one or two genes. It is recognized that a model based on multiple Mendelian factors can explain the continuous variation observed in quantitative traits (Figures 2.1B and 2.1C). For a completely additive system, increasing the number of genes responsible for differences in a given quantitative trait gives a binomial approximation for a normal distribution (Figure 2.1B). Quantitative traits often follow a normal distribution, which is described by a mean and a variance (Figure 2.1C). There are several phenomena that increase the complexity of quantitative traits expression and inheritance, and these are considered below.

The environment influences the expression of quantitative traits

Each genotype may have a norm of reaction (i.e. a range of phenotypes) instead of a single unique expression. Therefore, the same genotypes can have different phenotypes, based on the environmental influence (Figure 2.2).

FIGURE 2.2
Norm of reaction of two genotypes (AA, aa) in different environments



A quantitative trait is usually a composite of many traits, which are governed by many genes with different effects (e.g. grain yield and its components)

Under simultaneous segregation of many genes affecting the trait, the number of possible genotypes increases with the number of loci. If these loci have small effects, distinguishing between the different genotypic classes is difficult. Because the phenotype is the final effect of different loci acting together, many genotypes may have the same phenotype. Under the same assumptions, an increasing number of loci would result in an increasing number of genotypes with the same phenotype.

Furthermore, there are different types of genetic effects among alleles, both within and among loci (i.e. additive, dominance and epistatic effects), which further increase the genetic complexity of quantitative

TABLE 2.1
Number of different genotypes in an F_2 population for a variable number of segregating loci with two alleles

Number of loci	Number of genotypes
1	3
2	9
3	27
5	243
10	59049
...	...
N	3^n

TABLE 2.2
Phenotypic values, genotypes and number of genotypes per phenotypic value (assuming two loci with two alleles each (A/a and B/b) with alleles A and B adding one unit to the final phenotypic value)

Genotype	Phenotypic Value	Number of genotypes per phenotype
AABB	4	1
AaBB, AABb	3	2
AAbb, AaBb, aaBB	2	3
Aabb, aaBb	1	2
Aabb	0	1

traits. Additive effects are the average effect of alleles, which are associated with the number of copies of each allele. Dominance effects originate from the interaction between (among) alleles at the same locus. For a single locus in a diploid species, the comparison between heterozygous loci (e.g. A_1A_2) with the parental homozygous loci (e.g. A_1A_1 and A_2A_2) determines the level of dominance: no dominance (i.e. $A_1A_2 = (A_1A_1 + A_2A_2)/2$), partial dominance (i.e. A_1A_2 is between $(A_1A_1 + A_2A_2)/2$ and A_1A_1 or A_2A_2), complete dominance (i.e. $A_1A_1 = A_1A_2$ when allele A_1 is dominant over A_2), and overdominance (i.e. $A_1A_2 > A_1A_1$ or A_2A_2). Quantitative traits are therefore regulated by many genes having diverse types of genetic effects. More details about genetic effects and their influence in genetic variation and breeding are given by Falconer and Mackay (1996) and Bernardo (2002).

The expression of individual genes is often modified by the expression of other genes (i.e. epistasis)

Epistasis is the interaction between alleles from different genes (i.e. interloci or non-allelic genetic interaction) (Holland, 2001). For two loci, epistasis is the failure of a gene replacement at one locus to remain the same when a gene is replaced at the other locus. Epistasis has strong consequences in plant breeding. First, the consideration of an allele as 'favourable' or 'unfavourable' may depend on the genotype at other loci. Therefore there are favourable and unfavourable combinations of alleles that breeders select for. Epistasis affects the average effects of alleles and dominance deviations and, consequently, the additive and dominance genetic variances (see Section 2.2.2). If epistasis is strong, there can be more heritable variance within lines during selfing and line development than expected. Second, epistasis reduces the correlation between the expression of quantitative traits of early and later selfing-generations. With the presence of epistasis, early generation testing and selection is expected to be less effective than delaying selection until later generations when epistatic effects (e.g. additive × additive epistatic effects) are fixed within lines. Third, epistasis contributes to heterosis and inbreeding depression, although in different manners. While hybrids and population-cross cultivars can exploit all forms of epistasis (additive × additive, additive × dominant and dominant × dominant), only dominance and dominance × dominance epistasis contribute to inbreeding depression (Holland, 2001). Inbreeding depression is heterosis in reverse only when epistasis is absent, but not in the presence of epistasis.

In summary, continuous variation for a quantitative trait is the result of the effect of multiple genetic factors and the environment. Hence, many genotypes can have the same phenotype, and the same genotype can have different phenotypes. The number of loci affecting a quantitative trait, their genomic position, their effects in the final phenotype, the interactions among them, their regulation and the effect of the environment in gene expression is largely unknown for most of quantitative traits (Bernardo, 2002).

2.2.2 Means, variances and correlations for quantitative traits

Genotypic value (G) is the value of a genotype for the trait under consideration. Classically in quantitative genetics, G has been divided into additive, dominance and epistatic effects: G = A + D + I (Falconer and Mackay, 1996). The variation among genotypic values in a breeding population is the genotypic variance (σ_G^2). The breeding value of one individual assesses its usefulness in selection. It is determined by the mean of its progeny and is associated with additive effects. Breeding value in one individual is twice the mean deviation of its outcrossed progeny from the population mean, which is equal to the sum of the average effects of the alleles it carries. The variation among breeding values is attributed to the additive effects of genes and is called additive genetic variance (σ_A^2). Dominance deviation, D, is the difference between the genotypic value (G) and the breeding value (A) for a given genotype in the absence of epistasis. The dominance deviations are due to within-locus interaction between the different alleles. Variation among genotypes for dominance deviations is the dominance genetic variance (σ_D^2). Finally, the variation associated with differences among genotypes for epistatic interactions is the epistatic variance (σ_I^2).

Genetic variance is the sum of the additive, dominance and epistatic genetic variance components: $\sigma_G^2 = \sigma_A^2 + \sigma_D^2 + \sigma_I^2$ (Hallauer and Miranda, 1988). The estimation of additive, dominance and epistatic effects requires knowledge of the allele composition of genotypes and their corresponding genotypic values. The estimation of genetic variance components is conducted using genetic mating designs with a family structure of relatives with known genetic covariance (see Section 2.2.6). Their estimates are used to assess heritability and expected response to selection.

The characterization of genetic properties of quantitative traits (loci effects, intra-locus gene action, epistasis, pleiotropy, linkage, allele frequencies, environmental influence in gene expression, etc.) has been pursued through the study of observable phenotypic properties (mean, variance, resemblance between relatives, correlation among traits, response to selection, inbreeding depression and heterosis). More recently, advances in plant genomics and molecular biology and physiology are contributing to better understand the genetic architecture of quantitative traits and facilitating the connection between genetic and phenotypic properties (Cooper, Podlich and Smith, 2004).

Observable phenotypic properties can be estimated statistically. For two traits (X and Y) measured in several experimental units (i.e. *n* genotypes), means, variances, covariance and correlation can be estimated as follows:

Means:

$$\bar{X} = \frac{1}{n}\sum_{i=1}^{n} x_i \qquad \bar{Y} = \frac{1}{n}\sum_{i=1}^{n} y_i$$

Variance:

$$\hat{\sigma}_x^2 = \frac{1}{n-1}\sum_{i=1}^{n}\left(x_i - \bar{X}\right)^2 = \frac{1}{n-1}\left(\sum_{i=1}^{n} x_i^2 - n\bar{X}^2\right)$$

Covariance:

$$\hat{\sigma}_{XY} = \frac{1}{n-1}\sum_{i=1}^{n}\left(x_i - \bar{X}\right)\left(y_i - \bar{Y}\right) = \frac{1}{n-1}\left(\sum_{i=1}^{n} x_i y_i - n\bar{X}\bar{Y}\right)$$

Phenotypic correlation:

$$r_{XY} = \frac{\hat{\sigma}_{XY}}{\hat{\sigma}_X \hat{\sigma}_Y}$$

Genotypic correlation:

$$r_G = \frac{Cov_G}{\sigma_{G_X}\sigma_{G_Y}} = \frac{\hat{\sigma}_{G_{XY}}}{\hat{\sigma}_{G_X}\hat{\sigma}_{G_Y}}$$

Heterosis is the superior performance of crosses relative to their parents (Falconer and Mackay, 1996). Mid-parent heterosis is the difference between the hybrid and the mean of the two parents (commonly expressed as a percentage):

$$\frac{\bar{X}_{F1} - MP}{MP} * 100 = \text{percentage Mid-parent heterosis}$$

where: $\bar{X}_{F1}$ is the mean of the hybrid; and MP the average of the two parents.
Mid-parent heterosis also may be defined as:

$$\bar{X}_{F1} - MP = \sum_{i=1}^{n} f_i^2 d_i$$

where: d_i is the level of dominance (deviation of the heterozygous from the homozygote mid-parent); and f_i is the difference in allele frequencies among the parents for locus i (Falconer and Mackay, 1996).

Heterosis is dependent on the presence of directional dominance and allele frequency differences. Heterotic groups in cross-pollinated species have been created and enhanced by creating groups and families that differ in allele frequencies in genes affecting target trait(s) (i.e. this increases the value of f_i in the above formula).

Inbreeding comes from mating individuals that are related by ancestry. The consequence of inbreeding is an increase in homozygosity that leads to a depressive negative expression of traits, referred to as inbreeding depression. Inbreeding depression can be expressed as:

$$\bar{X}_0 - \bar{X}_F = 2pqFd$$

where: $\bar{X}_0$ and $\bar{X}_F$ are the mean of the population without and with inbreeding, respectively; p and q are the allele frequencies in the populations; F is the inbreeding coefficient; and d the dominance deviation (Falconer and Mackay, 1996).

The estimation and expression of inbreeding depression (ID) can be calculated as: $\bar{X}_0 - \bar{X}_F$ as the ID in absolute units, where $\bar{X}_0$ is the mean of the trait without inbreeding and $\bar{X}_F$ is the mean of the trait with a given amount of inbreeding F ($0<F<1$).

Inbreeding depression is commonly reported as a percentage:

$$\frac{(\bar{X}_0 - \bar{X}_F)}{\bar{X}_0} * 100 .$$

2.2.3 Genetic linkage and implications in plant breeding

Loci located in different chromosomes are assorted independently. However, loci close together in the same chromosome are not assorted independently: they tend to be inherited together and are considered 'linked loci'. Groups of alleles in loci that are linked and are transmitted together from one generation to the next are called linkage blocks or haplotypes (Figure 2.3). When considering two loci, linkage can be in coupling or repulsion (Falconer and Mackay, 1996). The degree of linkage is measured by the recombination frequency, which determines the proportion of recombinant and parental gametes. If 'R' is the genetic recombination between loci A and B, the gametes frequencies are estimated as shown in Table 2.3.

Genetic linkage has important implications in plant breeding:

TABLE 2.3
Gametic frequencies for two loci with two alleles each (A/a and B/b)

Gamete	Linked		Unlinked
	Coupling	Repulsion	
AB	½ (1-R)	½ R	¼
Ab	½ (1-R)	½ R	¼
Ab	½ R	½ (1-R)	¼
aB	½ R	½ (1-R)	¼

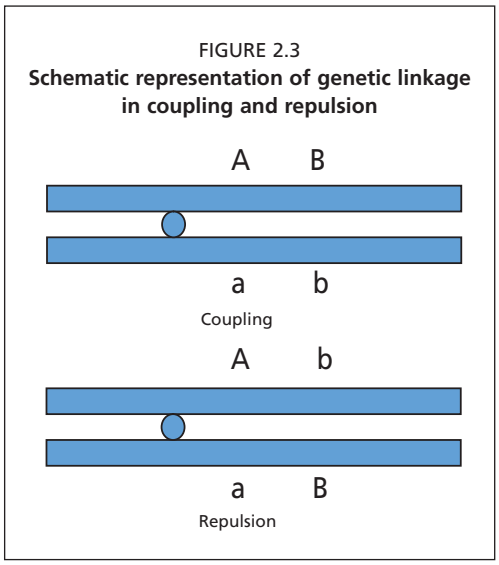


FIGURE 2.3
Schematic representation of genetic linkage in coupling and repulsion

- Selection during breeding is actually applied to linked blocks of genes housing target genes regulating the expression of target traits.
- Linkage is desirable in breeding if favourable or unfavourable alleles are linked in coupling, and undesirable if they are linked in repulsion. The probability of obtaining desirable genotypes in a segregating population is greater than with independent assortment when desirable alleles are in coupling.
- If the genes are in repulsion, large segregating populations will be necessary to break unfavourable linkages by recombination. Heterozygosity is necessary to break up linked genes; otherwise, crossing over does not create new combinations of alleles at linked genes.
- The probability of maintaining desirable linkage blocks in backcross breeding populations is higher than with F_2 breeding populations. At the same time, the probability of breaking up unfavourable linkage blocks is greater in F_2 populations than in backcross populations.

Additive and dominance variance estimates are biased by linkage. Additive variance, σ^2_A, increases with coupling and decreases with repulsion. Dominance variance, σ^2_D, increases in both types of linkage if dominance effects of the linked loci have the same sign. As a consequence, the degree of dominance
(dominance ratio estimated as $\sqrt{4\sigma^2_D / 2\sigma^2_A}$)
increases with repulsion (i.e. pseudo-dominance). This pseudo-dominance decreases with recombination (e.g. applying random mating).

2.2.4 Mating system and population structure

Crop plants are propagated asexually, sexually or both. The mode of reproduction directly affects the population structure of breeding and natural populations of crop species. Asexual reproduction involves either vegetative propagation or apomixis. The cultivars in asexually propagated crops are clones or mixtures of clones. Selection can be conducted among clones to select those most suitable, which are then propagated asexually. Crops that reproduce sexually are self-pollinated, cross-pollinated or a combination. Self-pollination induces reduction of heterozygosity and fixation of alleles in a homozygous condition. Therefore populations and landraces (local or traditional varieties) of self-pollinated crops are commonly a mixture of inbreds, while cultivars are commonly selected pure lines. In contrast to self-pollination, cross-pollination maintains heterozygosity if the populations are big enough and plants mate at random. Therefore populations of cross-pollinated species are a mixture of hybrid genotypes, while cultivars are commonly hybrids or synthetics developed from crossing selected inbreds, or

selected subpopulations from broad-based populations (e.g. pearl millet). Average heterozygosity is much greater in cross-pollinated species than in self-pollinated species. Selfing induces inbreeding depression while cross-pollination induces hybrid vigour. The degrees of inbreeding depression and hybrid vigour are greater in cross-pollinated crops than in self-pollinated crops.

Most of the scenarios in quantitative genetics are studied in populations of genotypes. These populations can be characterized by their genotype and allele frequencies. In large, random-mating populations the gene frequencies and the genotype frequencies have a simple relationship ($p_{ij} = p_i p_j$) and are constant from generation to generation. A population with these characteristics is in Hardy-Weinberg equilibrium (Falconer and Mackay, 1996) and is frequently used as reference framework in Quantitative Genetics modelling. There are several events that cause populations to deviate from an idealized Hardy-Weinberg equilibrium: mutation, genetic drift, migration and selection. Most of these forces are frequently acting together in breeding populations. Small population sizes cause unpredictable alterations in allele frequencies, known as genetic drift. Consequences in breeding are that genetic variation is reduced, and desirable alleles can be lost by chance. Migration can also be used in crops plants through the introduction or introgression of genotypes with increased frequencies of desirable alleles. Selection is the most powerful directional force to change allele frequencies and consequently the expression of quantitative traits. The response to selection depends on allele frequencies of loci regulating the expression of target traits, heritabilities and selection intensities, and the breeding approaches employed.

When considering allele and genotypic frequencies at several loci together there is another type of disequilibrium, known as gametic phase or linkage disequilibrium (LD). LD is defined as the non-random association of alleles at different loci (Bernardo, 2002). For two loci, it is measured as the difference between the observed gamete frequencies and the product of the frequencies of the corresponding alleles:

$$D = p_{(A_iB_j)} - p_{(A_i)}p_{(B_j)}$$

where $p_{(A_iB_j)}$ is the frequency for the A_iB_j gamete, and $p_{(A_i)}, p_{(B_j)}$ the allele frequencies for alleles A_i and B_j, respectively.

High LD exists whenever there is linkage or the population is subject to selection, genetic drift or admixture. It is greatly influenced by several factors, such as population structure, recombination hot spots and the mating system. Genetic recombination between loci in disequilibrium reduces LD. Therefore, LD between unlinked loci in different chromosomes decreases faster than LD between linked loci ($D_t = D_0 (1\text{-}r)^t$, where r = recombination frequency, t = number of generations and D = amount of LD). The tighter the linkage, the longer LD is maintained.

In recent years LD has been used or exploited to associate genomic regions with the expression of quantitative traits, either through artificial LD created by hybridization of contrasting genotypes (QTL mapping) or by using naturally occurring LD in breeding or natural populations (i.e. association genetic studies) (Lee, 1995; Buckler and Thornsberry, 2002; Mackay and Powell, 2007).

2.2.5 The environment and its interaction with genotypes in plant breeding

The environment affects the expression of quantitative traits, and different environments can affect genotypes differently (See Chapter 20). Phenotypic values are classically divided into genotypic (G), environmental (E) and genotype × environmental interaction (G×E) effects: P = G + E + G×E. Likewise, phenotypic variance is divided into genotypic, environmental and G×E variance components: $\sigma_P^2 = \sigma_G^2 + \sigma_E^2 + \sigma_{GE}^2$. In breeding, several genotypes are commonly evaluated in several environments. When genotypes tested differ in their relative performance across environments there is G×E, which can affect response to selection. Non-crossover interaction, i.e. where rank of genotypes does not change across environments, does not have any effect in selection as the best and worst genotypes are the same in all locations. Crossover interaction, where the rank of genotypes changes across environments, has strong consequences in breeding as best and worst genotypes in different environments are not the same (Romagosa and Fox, 1993). There are different breeding strategies that deal with this issue (Annicchiarico, 2002, and see Chapter 20 in this volume). Definition of target and selection environments is critical when allocating resources in a breeding programme. In this process, several decisions have to be made regarding selection for broad versus specific or local adaptation; selection on farm versus research station; and selection under optimal versus stressed conditions (Atlin, Cooper and Bjørnstad, 2001). Cultivars can perform well under a wide range of environments (broad adaptation) or under specific growing conditions (narrow, specific or local adaptation) (Ceccarelli, 1989).

Selection response in the target environment can be expressed as:

$$R_T = \sigma_G i h_s r_G$$

where σ_G = genetic standard deviation, i = selection intensity, h_s = square root of heritability in the selection environment, and r_G = genetic correlation between the selection and target environments (adapted from Bänziger and Cooper, 2001). Thus the effectiveness of a selection environment is determined by the heritability of the traits(s) under selection in that environment, and the genetic correlation between the performance in the selection environment and the target environment (i.e. indirect selection theory, see Section 2.2.8). When the heritability is low or the correlation is low or negative, or both, little progress in the target environment can be expected regardless of who does the selection (farmers or breeders). Multi-environment trials conducted at a large number of sites to adequately sample the target environment maximizes the correlation between target and selection environments, facilitates selection of broadly adapted hybrids or varieties, and exploitation of G×E. Weighted selection strategies, where individual trials found more relevant to the target environments are given more emphasis than less relevant trials, can be used (Podlich, Cooper and Basford, 1999). Increasing the number of environments, as the number of entries decreases during the breeding process, evaluating in environments that disclose genetic variation for the traits under selection, and combining the understanding of the genetic control of target traits and of the target environments, are important components of successful breeding strategies. As we will see later in the book, Participatory Plant Breeding

makes it easier to implement the first and the third of these strategies.

2.2.6 Heritability

Heritability is the relative importance of genetic and non-genetic factors in the expression of phenotypic differences among genotypes in a population (Fehr, 1987). There are two basic types of heritability: broad-sense heritability and narrow-sense heritability (Holland, Nyquist and Cervantes-Martinez, 2003; Nyquist, 1991).

Heritability in the broad sense (H) is the proportion of the phenotypic variance of family means that is due to all genetic effects (Falconer and Mackay, 1996; Holland, Nyquist and Cervantes-Martinez, 2003): $H = h^2_b = \sigma_G^2/ \sigma_P^2$. Broad-sense heritability can be estimated from standard analysis of variances. For example, in the case of genotypes evaluated across several environments, the corresponding analysis of variance and heritability estimate can be illustrated in Table 2.4.

Genotypic and phenotypic variance components can also be estimated using Restricted Maximum Likelihood methods (Holland, Nyquist and Cervantes-Martinez, 2003).

Heritability in the narrow sense (h^2) is the proportion of phenotypic variance among individuals in a population that is due to heritable genetic effects (Nyquist,

TABLE 2.4
Analysis of variance and broad sense heritability estimates in the case of a series of g genotypes evaluated across e environments

Source of variation	Degrees of freedom	Mean Squares	Expected Mean Squares
Environment	e-1		$\sigma_e^2 + g\sigma_{r(E)}^2 + r\sigma_{GE}^2 + rg\sigma_E^2$
Rep(Environment)	(r-1)e		$\sigma_e^2 + g\sigma_{r(E)}^2$
Genotype	g-1	MS_G	$\sigma_e^2 + r\sigma_{GE}^2 + re\sigma_G^2$
Genotype × Environment	(g-1)(e-1)	MS_{GE}	$\sigma_e^2 + r\sigma_{GE}^2$
Error	(g-1)(r-1)e	MS_E	σ_e^2

Notes:

Total phenotypic variance: $Var(Y_{ijk}) = \hat{\sigma}_P^2 = \hat{\sigma}_e^2 + \hat{\sigma}_{GE}^2 + \hat{\sigma}_G^2$

Phenotypic variance of genotypic means: $Var(\overline{Y}_{ijk}) = \hat{\sigma}_{\overline{P}}^2 = \frac{\hat{\sigma}_e^2}{re} + \frac{\hat{\sigma}_{GE}^2}{e} + \hat{\sigma}_G^2 = \frac{MS_G}{re}$

Genotypic variance $= \hat{\sigma}_G^2 = (MS_G - MS_{GE})/re$

Heritability on individual experimental unit basis: $H_i = h_{bi}^2 = \frac{\hat{\sigma}_G^2}{\hat{\sigma}_G^2 + \hat{\sigma}_{GE}^2 + \hat{\sigma}_e^2}$

Heritability on a genotypic-mean basis: $H_m = h_{bm}^2 = \frac{\hat{\sigma}_G^2}{\hat{\sigma}_G^2 + \frac{\hat{\sigma}_{GE}^2}{e} + \frac{\hat{\sigma}_e^2}{re}}$

TABLE 2.5
Relatives, their covariance and regression or correlation values in terms of narrow sense heritability

Relatives	Covariance (w/o epistasis)	Regression (b) or correlation (t)
Parent – Offspring	½ σ^2_A	b = ½ h^2
Midparent - Offspring	½ σ^2_A	b = h^2
Half-sibs	¼ σ^2_A	t = ¼ h^2
Full-sibs	½ σ^2_A+ ¼ σ^2_D	t ≥ ½ h^2

TABLE 2.6
Additive and non-additive variance components in different generations

Generation	σ_A^2	σ_D^2	σ_{AD}^2	σ_E^2
P_1	0	0	0	1
P_2	0	0	0	1
F_1	0	0	0	1
F_2	1	1	0	1
BC_{11}	½	1	- 1	1
BC_{12}	½	1	1	1

Notes: $\sigma_A^2 = (2\sigma\ ^2_{F2} - \sigma\ ^2_{BC11} - \sigma\ ^2_{BC12})$;
$\sigma D2 = (\sigma\ ^2_{BC11} + \sigma\ ^2_{BC12} - \sigma\ ^2_{F2} - \sigma\ ^2_{P1/P2/F1})$;
$\sigma AD = ½(\sigma\ ^2_{BC12} - \sigma\ ^2_{BC11})$

1991; Holland, Nyquist and Cervantes-Martinez, 2003): $h^2_n = \sigma_A^2 / \sigma_P^2$. Narrow-sense heritability can be estimated from variance components or from parent-offspring regression. In both cases, genetic relationships among relatives (lineal (parent-offspring) or collateral (full- or half-sibs)) are used (Table 2.5). The estimation of additive and non-additive variance components is conducted through linear models and proper mating designs (e.g. North Carolina I, II and III, Hallauer and Miranda, 1988) or using information from different generations (Kearsey and Pooni, 1998) (Table 2.6).

Heritability is used to estimate expected response to selection and to choose the best breeding approach to improve the target trait(s). Traits with high heritabilities can be selected on a single-plant basis (e.g. mass selection), faster, and in a low number of environments. In contrast, traits with low heritabilities require selection on a family basis and in a greater number of environments to determine breeding values of genotypes. Heritability estimates for the same trait are variable (i.e. heritability of a trait is not a fixed value) and their magnitude depends on several factors (Fehr, 1987):

- ***Environment***: it is important to have adequate samples of environments from the target population of environments. In addition, estimates for genetic variance should be free of G×E variance.
- ***Reference population***: the amount of genetic variation and inbreeding present in the population affects heritability estimates. Higher inbreeding levels are associated with higher genetic variances and therefore with higher estimates of heritability.
- ***Sample of genotypes evaluated***: genotypes used to estimate heritabilities in one population should be chosen at random. If the sample is not a representative random sample (e.g. selected genotypes), the ratio between genetic and phenotypic variation is called *Repeatability* (Fehr, 1987). Repeatability estimates in a single environment provide a measure of how much of the variation is genetic and therefore is a measure of the degree of precision of data and the ability to detect significant differences among genotypes.
- ***Method of estimation***: heritability of a quantitative trait can be computed by several methods (variance components, parent-offspring regression, etc.) and heritability estimates can differ among them (e.g. heritability on a family basis is greater than on a plant basis; see Table 2.4). Heritability estimates calculated on the basis of selection unit are preferred to estimated expected response to selection.

- ***Generation or progenies***: different progenies exploit different proportions of additive and dominance variances. Inbred progenies have greater heritabilities than full-sib and half-sib families.
- ***Allele frequencies***: heritability is affected by allele frequencies. Therefore any change in allele frequencies (selection, genetic drift, mutation, migration) could change heritability values for the same trait and reference population.

Heritability of a trait can be estimated using the amount of genetic gain that is realized by selection within a population (Falconer and Mackay, 1996). This is known as *realized heritability* and can be estimates *a posteriori* as : h^2=R/S, where R = response to selection and S = effective selection differential applied in selection.

2.2.7 Response to selection

The theoretical response to selection can be defined as $\Delta G = S\ h^2$, where S is the selection differential (the difference between the mean of the selected individuals and the mean of the whole population) and h^2 the heritability of the target trait(s). S is determined by the intensity of selection (i), which is the number of genotypes selected relative to the total number under evaluation. Intensity of selection is the standardized selection differential:

$$i = \frac{S}{\sigma_P}$$

where σ_P is the square root of the phenotypic variance (Figure 2.4)

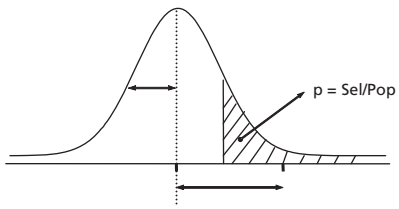
Alternative selection methods differ in the types of progenies evaluated and recombined, and the seasons required per cycle. Hence, Eberhart (1970) incorporated the number of years (y) and the parental control (c) into the prediction formula. After additional elaboration, a general expected genetic gain formula can be expressed as:

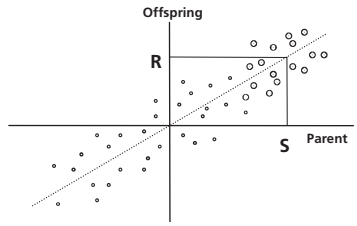
$$\Delta G = \frac{ic}{y}\frac{\sigma_G^2}{\sqrt{\sigma_P^2}} = \frac{ic}{y}\frac{\sigma_G^2}{\sqrt{\frac{\sigma_e^2}{re} + \frac{\sigma_{GE}^2}{e} + \sigma_G^2}}$$

where i = standardized selection differential, c = parental control (see below), y = seasons per cycle, r = number of replications per environment, e = number of environments, and σ_{Gl}^2, σ_{GE}^2 and σ_e^2 are as defined earlier. The variance components are estimated from the analysis of variance or mixed model solutions (Hallauer and Miranda, 1988; Holland, Nyquist and Cervantes-Martinez, 2003).

Increased heritability results in more effective selection. In the genetic gain formula, the value of the numerator can be increased by increasing the selection intensity, parental control or genetic variance. The value of the denominator can be decreased by decreasing the number of seasons per cycle or the phenotypic variance. Response to selection is greater when a lower proportion of individuals or families are selected. However, by decreasing the proportion selected (e.g. increasing selection intensity) the effective population size is reduced, thus increasing the possible occurrence of genetic drift or inbreeding. Desirable genes can be lost due to genetic drift. Because these effects can be detrimental for future gains, it is recommended to select a proportion of at least 20 percent. Parental control, c, can be increased by recombining genotypes where both sources of gametes originated in selected genotypes (c = 1). If the male gametes are coming from unselected genotypes, then c is 0.5. Hence, selection before pollination, where only selected genotypes contribute to the next generation, is more effective than selection after pollination, where non-selected geno-

FIGURE 2.4
Schematic representation of selection differential and response to selection





S = selection differential $S = x_{sel} - x_{pop} = i\sigma_p$

i = Standardized selection differential $i = S/\sigma_p = z/p$

$$R = b_{\bar{P}} S_o = h^2 S = i h^2 \sigma_p = \frac{i\sigma_g^2}{\sqrt{\sigma_p^2}} = \frac{c}{y}\frac{i\sigma_g^2}{\sqrt{\sigma_p^2}}$$

types can contribute to the next generation. Further increase of *c* can be accomplished by inter-mating selfed progenies of selected genotypes (*c* = 2). Genetic variance can be increased by selecting parents with high genetic diversity, increasing the degree of inbreeding before evaluation, increasing the recombination between cycles or using different types of progenies. Different types of progenies express—and therefore exploit—different proportion of genetic variances. The theoretical proportion of σ_A^2 of total σ_G^2 is 0.25 for half-sib progenies, 0.5 for full sib progenies and 1.0 for S_1 progenies. In addition to the type of progeny, different populations have different proportions of genetic variance components, and therefore different gains can be observed and expected.

The number of seasons required to complete a cycle can be reduced by using off-season nurseries at lower latitudes or in the opposite hemisphere, or by using greenhouses and growth chambers. The phenotypic variance can be decreased by increasing the number of replications and environments, or using improved statistical design and analysis techniques that reduce the error variance and more accurately estimate progeny performance. Increasing the number of environments affects selection response more than increasing the number of replications. However, the environments have to be representative of the target area because if the environments are very different, σ_{GE}^2 can increase substantially and thereby reduce selection gain. This is the case when, for example, the relative importance of stress factors differs between selection and target environment (Betrán, Bänziger and Menz, 2004; Cooper *et al.*, 2006).

Proper field experimental design and analysis (see also Chapters 3 and 20 in this volume), field stratification, number of plants measured, uniform soils and treatments, and reliable data collection and processing reduce the error term and subsequently increase the gain. Several field experimental designs are used in plant breeding. The most common are randomized complete block, incomplete lattices and, more recently, row-and-column designs among replicated designs, and augmented designs among unreplicated designs. Optimal experimental designs and field layout are useful in reducing the error, increasing the precision of mean estimates, and consequently improving selection (Gilmour, Cullis and Verbyla, 1997). Data processing

and analysis has improved in recent years, with more powerful computer hardware and software addressing G×E interaction of multi-environment trials, in-trial spatial variation, correlation among traits, variance components estimation, prediction of genetic parameters, QTL mapping, MAS, etc. (van Eeuwijk *et al.,* 2005; Romagosa, van Eeuwijk and Thomas, 2008).

The use of artificial inoculation with pathogens and infestation with major pests has increased display of genetic variation and the heritability and gain for host plant resistance. Similarly, the use of managed drought, nitrogen or low-pH stress environments has increased heritability and selection gain for abiotic stress tolerance (Bänziger and Cooper, 2001).

Recent developments in biotechnology mean that effectiveness of selection can be increased by the use of molecular tools. For example, molecular markers can be used to: (1) increase selection intensity while selecting genotypes with different genetic backgrounds to maintain genetic variance; (2) select before pollination genotypes with the desired allele composition for markers associated with traits of interest; (3) conduct selection in environments not representative of the target area (e.g. off-season nurseries); and (4) conduct MAS *per se* or in combination with phenotypic selection.

2.2.8 Correlated response to selection and indirect selection

Several relevant traits are often considered simultaneously in plant breeding, particularly when selection is done by farmers. The relationship among them determines breeding strategies and response to selection. The association among desirable traits can be negative (e.g. increasing grain yield is associated with lower protein content in maize (Duvick and Cassman, 1999)), or positive (e.g. reduced anthesis–silking interval under drought is associated with increased yield in maize (Edmeades, Bolaños and Chapman, 1997)). Correlations among traits can be due to pleiotropy (same loci affect both traits), linkage/linkage disequilibrium (different loci affect the traits but these loci are linked together), or environmental effects. Linkage in coupling will cause positive correlation and negative correlation in repulsion. If the environment affects both traits in the same way (e.g. plant height and grain yield in maize), it can create a positive correlation. Different types of correlations can be calculated: phenotypic correlations (r_P) are calculated using phenotypic values; genotypic correlations (r_G) are calculated using genotypic values; and additive genetic correlations (r_A) (also known as genetic correlations) are calculated using breeding values (Falconer and Mackay, 1996). Genetic correlations among traits can change with selection as a consequence of change in allelic frequencies.

If the direct selection response for trait X is $R_X = ih_X\sigma_{AX}$, the correlated response in trait Y is defined as $CR_Y = ih_X h_y r_A \sigma_{PY}$, where h_x and h_y are the square roots of heritabilities for traits X and Y, respectively; r_A is the genetic correlation between traits X and Y; i is the selection intensity; and σ_P is the phenotypic variance. Indirect selection for trait Y can improve trait X. Therefore the relative efficiency or merit of indirect selection can be compared with the direct selection for trait X as follows:

$$\frac{CR_X}{R_X} = \frac{i_Y h_Y r_A \sigma_{AX}}{i_X h_X \sigma_{AX}} = \frac{i_Y h_Y r_A}{i_X h_X}$$

(Falconer and Mackay, 1996)

Indirect selection can be more effective than direct selection when this ratio is >1 in cases where secondary traits show greater heritabilities than the primary trait

and high correlations between secondary and target traits are present, or if greater selection intensities can be applied to the secondary trait (e.g. easier to screen in big populations). If selection intensity in both traits were considered the same, indirect selection would be superior to direct selection when $r_A h_y$ is greater than h_x. Ideally, a secondary trait should be associated genetically with the target trait, highly heritable, easy and fast to measure, non-destructive, stable over the measurement period, and/or observable at or before flowering so that undesirable parents are not crossed (Edmeades, Bolaños and Chapman, 1997).

Selection indices combine information from different traits with the goal of selecting genotypes with the highest aggregate breeding value. Many breeders and especially farmers have an ideotype in mind when applying selection, which is a rather subjective application of a selection index. More objective selection indices are linear combinations of observable trait values that maximize the expected genetic gain in an aggregate breeding value. Baker (1986) and Lin (1978) have reviewed the theory of selection indices and their application to plant breeding.

MAS (i.e. selection of genotypes based on molecular markers associated with target traits) is also a form of indirect selection. MAS for quantitative traits can be used in situations where phenotypic selection can not be conducted (e.g. off-season nurseries), when target traits have low heritability and there are tight linkage between QTLs and markers, and a high proportion of additive variance is explained by the markers (Lande and Thompson, 1990; Hospital and Charcosset, 1997).

2.3 KEY EXPERIMENTS FOR RESPONSE TO SELECTION

Selection experiments have been designed to make the improvement of quantitative traits more efficient, trying to maximize as much as possible the additive effects, as well as to gather genes with complementary dominant and epistatic effects in genotype crosses and synthetic varieties. There are many examples that show the efficacy of these experiments. Below, we discuss the results of some selection experiments for complex traits published in the last years, which supposedly reveal the genetic architecture of quantitative traits. Experiments are divided into short-term, mid-term and long-term recurrent selection experiments. We are focusing this section on recurrent selection experiments because they reveal better than any other kind of selection method the role of the hidden genetic factors responsible for performance and behaviour of quantitative traits. Experiments that combine selection and subsequent recombination of selected genotypes permit the best exploration of the intricate assortment of both major genes and genes with small effect for the trait studied.

Recurrent or cyclic selection is generally applied to a population, where loci responsible for a quantitative trait are segregating with different gene effect and at different allele frequency, except for the case of an F_2 population derived from the cross of two inbred lines, in which allele frequencies for all segregating loci are 0.5. The aim of recurrent selection programmes is to increase progressively, cycle after cycle, the frequency of the favourable alleles responsible for the performance of the trait under selection (see later Chapters). The general scheme of each selection cycle involves three steps: (1) creation of a family structure from selection units; (2) evaluation

of familial test units in replicated trials in different environments, i.e. progeny testing; and (3) recombination of genotypes related to the families selected (recombination units) in the evaluation trial (Moreno-González and Cubero, 1993).

The above general scheme may be modified for different methods of selection, increasing or reducing the number of generations. For example, the three steps are combined in one generation per cycle in the recurrent mass selection method, and in two generations for the half-sib or full-sib family selection method as first introduced by Vilmorin in 1856 for sugar beet. In the case of mass selection, the selection units are individual plants, rather than families. In the case of half-sib and full-sib selection, creation of new families and recombination of selected families is the same operation, thus both steps are done in the same season, while family evaluation is carried out on the next generation. In contrast, any of the basic steps may involve more than one generation. For example, (1) creation of a family structure in the S_2 family selection method requires two generations; (2) evaluation trials may be conducted during more than one year either to reduce the effect of genotype × year interaction or to conduct selection in multiple stages, such as genotypes selected at low selection intensity in the first year may be re-evaluated at higher selection intensities in following years (Piper and Fehr, 1987); and (3) selected genotypes may be recombined during more than one generation to break up repulsion linkages among favourable alleles.

Many recurrent selection experiments have been reported during the last three or four decades in the literature, which show genetic improvement for the selected trait in several crops. We choose here to discuss some of these experiments based on three criteria: (1) enough information is available to rely on the accuracy of results concerning genetic improvement; (2) comparison among different selection methods is possible; and (3) additional information is provided to help us to interpret the structure of genetic and environmental factors involved in complex traits. Short-term, mid-term and long-term selections are arbitrarily designed here as those experiments that have undergone <6, between 6 and 20, and >20 selection cycles, respectively.

2.3.1 Short-term recurrent selection experiments. The case of the BS11 population

A maize population, BS11 from Iowa State University, USA, has been studied for several factors affecting the process of selection for complex traits in short-term recurrent selection experiments (Weyhrich, Lamkey and Hallauer, 1998a, b; Guzman and Lamkey, 1999, 2000). These authors focus on two useful concepts for plant breeders, which are worth comment: (1) comparisons of the response of the same population to different intra-population and inter-population methods of selection; and (2) the role of the effective population size in the selection response and the genetic variability of the population.

Six intra-population methods—full-sib, half-sib, mass, modified ear-to-row, S_1 progeny and S_2 progeny selections—and one inter-population method, reciprocal full-sib recurrent selection (RFSRS), were applied to the same BS11 maize population and compared for grain yield and other traits during five selection cycles, except for half-sib selection, for which only four cycles were completed (Weyhrich, Lamkey and Hallauer, 1998a). General descriptions of the above and other schemes of selection methods may be found in Moreno-

González and Cubero (1993) and in Chapters 5 to 13 in this volume. Averaged selection responses for the grain yield trait in the populations *per se* and the population testcrosses are summarized in Table 2.7. Improved populations were testcrossed to the original BS11 population and to inbred B79. The S_2 progeny selection method resulted in the highest genetic gain per cycle for both the population *per se* (4.5 percent relative to the original cycle) and the average over population testcrosses (3.3 percent), followed by the modified ear-to-row selection method, which showed a genetic gain of 3.6 and 2.7 percent for the population *per se* and the testcross average, respectively (Weyhrich, Lamkey and Hallauer, 1998a). The superiority of the S_2 progeny selection over the other methods in this experiment was attributed to the importance of additive effects relative to dominance effects in the BS11 population. In contrast, other reports had found that testcross or reciprocal selection methods were superior to inbred progeny methods, probably because the presence of non-additive relative to additive effects was important in those populations studied (Horner, Magloire and Morera, 1989; Lamkey, 1992; Holthaus and Lamkey, 1995a,b).

In addition, the S_1 progeny selection method was applied to the BS11 maize population during five cycles, using four different effective population sizes (i.e. recombination of 5, 10, 20 and 30 selected lines) but with a common selection intensity of 20 percent for all of them (Guzman and Lamkey, 2000). Comparison of the original population (C_0) and selection cycle five (C_5) showed that genetic gain for grain yield of C_5 relative to C_0 was significant for all effective population sizes, with the highest yield gain for effective population size 10, followed by population sizes 30, 5 and 20. No significant difference was found among the additive genetic variance and heritability estimates of the four C_5 effective population sizes and population C_0. The results of this study showed that (1) use of smaller population size would not limit genetic progress in short-term recurrent selection; and (2) there is no clear advantage to using a larger population size to maintain genetic variability in short-term selection experiments (Guzman and Lamkey, 2000).

2.3.2 Mid-term recurrent selection experiments

A summary of the genetic gains for several cycles of selection, selection methods

TABLE 2.7
Grain yield genetic gains (%) averaged over cycles for different recurrent selection methods in the maize population BS11

Selection method	Selection cycles (No.)	Genetic gain per cycle (%)	
		Population per se	Averaged over population testcrosses
Full-sib	5	1.4	1.6
Half-sib	4	1.6	2.1
Mass	5	0.6	0.5
Modified ear-to-row	5	3.6	2.7
RFSRS†	5	2.6	2.6
S1-progeny	5	1.9	1.6
S2-progeny	5	4.5	3.3

Notes: † = reciprocal full-sib recurrent selection.
Source: adapted from Weyhrich, Lamkey and Hallauer, 1998a.

TABLE 2.8
Genetic gains (%) averaged over cycles for different recurrent selection methods in the populations *per se* and population crosses, and the change in genetic variance through selection cycles

Crop and Trait	Selection method	Selected source population	Selection cycles (No.)	Genetic gain per cycle (%)		Change in genetic variance over cycles	References
				Population per se	Averaged population crosses		
Oat yield	$F_{4.6}$ lines	Univ. Minnesota	7	2.2		No change	De Koeyer and Stuthman, 1998
Spring wheat kernel weight	F_3 lines	Univ. North Dakota	8	4.5		No change	Wiersma *et al*., 2001
Maize yield	RFSRS†	BS10	5	3.2	2.5		Hallauer, 1984
	RFSRS	BS11	5	2.9	2.5		
Maize yield	RFSRS	BS10	7	2.0			Rodriguez and Hallauer, 1988
	RFSRS	BS11	7	0.8			
Maize yield	RFSRS	BS10	8	3.0	6.5		Eyherabide and Hallauer, 1991
	RFSRS	BS11	8	1.6	6.5		
Maize yield	RFSRS	BS10	10	2.7	1.6	No change	Frank and Hallauer, 1999
	RFSRS	BS11	10	2.3	1.6	No change	

NOTES: † RFSRS = reciprocal full-sib recurrent selection
Source: adapted from references.

and crops is shown in Table 2.8. Most of the experiments are for maize but two are reported for other crops.

Self-fertilized crop species

A recurrent selection programme to increase grain yield in oat has been carried out at the University of Minnesota, United States of America, since 1968. The selection method comprises three steps: (1) selection of 21 $F_{4.6}$ lines out of 630 tested; (2) creation of 63 F_1 single crosses by intercrossing the 21 selected lines, each with another six different lines; and (3) derivation of 10 lines from each F_4 (De Koeyer and Stuthman, 1998). The linear regression response to selection over seven cycles was 2.2 percent per cycle relative to the original population (Table 2.8). The results indicated that there has not been significant change in the estimates of the genetic variance through the seven selection cycles (De Koeyer and Stuthman, 1998).

A recurrent selection programme to increase kernel weight in spring wheat was initiated at Fargo, North Dakota, United States of America, in 1967, and moved to St. Paul, Minnesota, United States of America, after the fourth cycle (Wiersma *et al*., 2001). The selection scheme was similar to that described above for oat, but the number of evaluated and selected lines, the number of inter-crosses and the generation used for the derived lines were different. Kernel size increased linearly at 4.5 percent per cycle over eight cycles (Table 2.8), with an indirect increase in flour yield. Results indicated no clear trend towards a decrease in genetic variance. Results suggested that the trait is controlled by several genes with small effects (Wiersma *et al*., 2001).

The two above experiments are examples of the efficiency of recurrent selection for complex traits in self-fertilized crop species. Other reports of recurrent selection in autogamous plant species have also been published. Nine cycles of recurrent selection for groat-oil content in oat produced a linear increase in the groat (caryopsis) oil content of oat at a rate of 6.5 percent per cycle and non-decrease in the genetic variation (Frey and Holland, 1999). It seems that additive effects were predominant in

self-fertilized crops. Soybean and cotton populations have also undergone recurrent selections with similar schemes to those described above (Piper and Feher, 1987; Miller and Rawlings, 1967).

Inbred versus population tester in reciprocal recurrent selection experiments

Reciprocal recurrent selection (RRS), first designed by Comstock, Robinson and Harvey (1949), tries to alter two different genetically populations to improve their cross mean. The original method consists of the following steps: (1) individual plants from two populations, A and B, are selfed and at the same time crossed to 3 to 5 random plants from the reciprocal female tester population, B and A, respectively; (2) selection in each population is based on the performance of the testcross half-sib families; (3) remnant seed from the selected S_1 families are mated at random within A and B to form new cycles of the A and B populations. Russell and Eberhart (1975) proposed a modification of RRS (MRRS), suggesting to use as tester of population A an inbred line derived from or related to B, instead of the population B itself; reciprocally, the tester of B should be an inbred line derived from or related to A.

A programme to compare MRRS and RRS using the maize populations BS21 and BS22 was initiated in the maize breeding programme at Iowa State University in 1975 (Russell, Blackburn and Lamkey, 1992). After three cycles of selection, the populations *per se* and the population cross of the MRRS method showed less genetic response than populations selected under the RRS method (Russell, Blackburn and Lamkey, 1992). It should be specially noted that the improved population BS22(H99HI), which uses inbred H99 as tester in the MRRS method, had less genetic response, genetic variance and predicted genetic gain than the other populations involved in the study (Russell, Blackburn and Lamkey, 1992). Inbred A632 was the tester of selected population BS21(A632HI). It seems that the elite inbred H99 might have masked dominant, favourable alleles present in the BS22 population. Comstock (1979), using quantitative genetics theory, compared population improvement with both types of testers (i.e. inbred vs. population) based on change in allele frequency. He concluded that the inbred line tester was not superior to the reciprocal population tester. Furthermore, the population tester might be superior to the inbred tester in some situations, especially if overdominance and multiple peak epistasis are present in the populations. Likewise, Moreno-González and Grossman (1976) demonstrated that the theoretical genetic gain of the population cross was higher when a low-yielding population (i.e. smaller allele frequencies for the segregating loci) was used as population tester.

The expected change in allele frequency in the selected population (Δp_A) (Moreno-González and Grossman, 1976; Falconer, 1981) will be

$$\Delta p_A = \frac{i p_A (1 - p_A)\left[a + (1 - 2p_T)d\right]}{2\sigma_p}$$

where i is the selection intensity; p_A and p_T are the frequencies of more favourable allele in the selected and tester populations, respectively; a and d are the additive and dominance effects; and σ_p is the phenotypic standard deviation of testcross or half-sib families. The numerator of the above expression is expected to have the same value both when the tester is the reciprocal population B and when it is an inbred line randomly derived from B; however, σ_p is larger when the tester is an inbred line

(Comstock, 1979). Thus Δp_A is not expected to be higher for the inbred tester than for the reciprocal population tester.

The same populations, BS21 and BS22, and breeding selection methods, RRS and MRRS, were also evaluated after the sixth cycle (Menz Rademacher, Hallauer and Russell, 1999). Results from this study were essentially similar to that of Russell, Blackburn and Lamkey (1992) for cycle three. The grain yield response of the population cross, BS21 × BS22, was higher for RRS (4.4 percent per cycle) than for MRRS (1.6 percent per cycle), and also for the testcross direct responses of the MRRS method (2.8 and 1.6 percent per cycle for BS21 × A632 and BS22 × H99, respectively) (Menz Rademacher, Hallauer and Russell, 1999). It seems that the MRRS was less efficient than RRS for increasing the population cross BS21 × BS22. Efficiency of MRRS depends on the choice of the tester, which is related to the type of gene action involved in the complementary alleles between the tester and the selection populations. If a favourable allele with complete dominance is fixed in the inbred tester, frequency of this allele is not expected to increase in the selection population, whereas this frequency will increase if the allele is segregating in a population used as tester. Thus, a limitation exists in terms of increasing the frequency of dominant favourable alleles in the selection population when they are fixed in the inbred tester.

Reciprocal full-sib recurrent selection experiments

The RFSRS method was designed for maize yield selection by Hallauer and Eberhart (1970), and has been applied to the BS10 and BS11 maize populations at Iowa State University. This method has proven to be very efficient in increasing the genetic gain of both the populations *per se* and the population hybrid (Table 2.8; Hallauer, 1984; Rodriguez and Hallauer, 1988; Eyherabide and Hallauer, 1991; Frank and Hallauer, 1999). The direct selection response for grain yield per cycle in the cross between the two populations was significant for all reported studies of evaluation, but varied among the different studies, being 2.5, 6.5 and 1.6 percent per cycle when the first 5, 8 and 10 selection cycles were evaluated, respectively. Sampling of populations and different years and environments of evaluation might account for these differences. The indirect selection response of the populations *per se* was similar in the four studies reported (Table 2.8), and it was consistently higher in BS10 than in BS11. It seems that selection for the population hybrid increased the allele frequency of favourable alleles in BS10 itself at a higher rate than in BS11. No significant differences between cycle 0 and 10 were found for the grain yield genetic variances of the populations *per se* and the population hybrid (Frank and Hallauer, 1999). This suggests that further response to selection should be expected in the next cycles of RFSRS applied to BS10 and BS11.

Over fifty years of reciprocal recurrent selection experiments

A RRS programme has been conducted by the Cooperative Federal-State maize breeding programme at Iowa State University with the synthetic maize populations BSSS and BSCB1 since 1949 (Keeratinijakal and Lamkey, 1993a), the year when the RRS method was first published (Comstock, Robinson and Harvey, 1949). Several reports on the genetic improvement in grain yield and other traits have been published through all the history of the RRS programme (Eberhart, Debela and Hallauer, 1973; Smith, 1983; Oyervides-

TABLE 2.9
Average genetic gains (%) of grain yield over cycles for the reciprocal recurrent selection (RRS) method applied to the maize populations BSSS and BSCB1 in the populations per se, population cross and population topcrosses

Selected population	Cycle No.	Genetic gain per cycle (%)			Change in genetic variances	References
		Population *per se*	Population cross	Population topcross		
BSSS	5	-0.1	4.1	1.2		Eberhart, Debela and Hallauer, 1973
BSCB1	5	1.0	4.1	0.3		
BSSS	7	2.2	4.3			Smith, 1983
BSCB1	7	0.7	4.3			
BSSS	8	1.9		1.4		Oyervides-Garcia and Hallauer, 1986
BSSS	10	1.3				Rodriguez and Hallauer, 1988
BSCB1	10	-1.6				
BSSS	9		6.1		Increase in additive and no change in dominance genetic variances of population cross	Betrán and Hallauer, 1996a, b)
BSCB1	9		6.1			
BSSS	11	1.7	6.9	2.8		Keeratinijakal and Lamkey, 1993a
BSCB1	11	1.9	6.9	3.9		
BSSS	11	2.6			No change in additive and reduction in dominance variance	Holthaus and Lamkey, 1995a

Source: adapted from references.

Garcia and Hallauer 1986; Rodriguez and Hallauer, 1988; Helms, Hallauer and Smith, 1989; Keeratinijakal and Lamkey, 1993a,b; Holthaus and Lamkey, 1995a,b; Betrán and Hallauer, 1996a,b). Some of the results for grain yield are summarized in Table 2.9. The direct response to selection in the population cross was reported to be very effective in four independent studies, 4.1 percent per cycle (Penny and Eberhart, 1971), 4.3 percent per cycle (Smith, 1983), 6.1 percent per cycle (Betrán and Hallauer, 1996a) and 6.9 percent per cycle (Keeratinijakal and Lamkey, 1993a), when the first 5, 7, 9 and 11 selection cycles were evaluated, respectively. However the observed indirect response to selection was much smaller in the populations *per se* than in the population cross, ranging from -1.6 percent per cycle in BSCB1 (Rodrigez and Hallauer, 1988) to 2.6 percent per cycle in BSSS (Holthaus and Lamkey, 1995a).

Part of the lower selection response in the populations *per se* compared to the population cross can be attributed to the genetic drift caused by restricted effective population size during the process of selection. The inbreeding coefficient progressively increases in the populations *per se* when the number of selected lines used for recombination has been small (i.e. <20) during several selection cycles. The expected inbreeding coefficient of a diploid population reproduced with a finite number of selected individuals during t generations can be estimated as:

$$F_t = 1 - (1 - F_0)\prod_{i=1}^{i=t}(1 - \frac{1}{2N_i})$$

where F_0 and F_t are the inbreeding coefficients of the original population and after t generations of selection, respectively; and N_i is the number of individuals selected at generation i.

If $F_0 = 0$, and N_i is constant (i.e. N) for all generations, the above equation becomes the known formula:

$$F_t = 1 - (1 - \frac{1}{2N})^t$$

In this case, if $N = 10$ and $t = 14$, then $F_{14} = 0.512$, which is larger than after one generation of selfing. Thus, inbreeding depression may become evident in these selected populations if dominance effects controlling the trait under selection (i.e. grain yield) were important. When responses were adjusted for effects of genetic drift, the improvements of the populations *per se* were similar to those of the population cross in the study of Keeratinijakal and Lamkey (1993b), and larger than in Smith's study (1983). In addition, additive and dominance effects were found in BSSS, but only dominance effects were important in BSCB1. The presence of important dominance effects may explain the inbreeding depression of the populations *per se*.

For grain yield, the additive genetic variance of population BSSS did not decrease after 11 cycles of RRS, whereas the dominance genetic variance was reduced, and the heritability estimates increased in BSSS when cycle 11 was compared to cycle 0 (Holtaus and Lamkey, 1995a). Likewise for grain yield, the additive genetic variance of the population cross BSSS × BSCB1 increased after nine cycles of RRS, with no reduction in the dominance genetic variance, and an increase in the heritability estimates (Betrán and Hallauer, 1996a). Therefore, it seems that the response of these populations to RRS will continue in the next cycles of selection.

Restriction fragment length polymorphism (RFLP) loci were used to determine changes in allele frequency, expected heterozygosity, and genetic variation in the populations BSSS and BSCB1 after 12 cycles of RRS (Labate, Lamkey and Woodman, 1999). Allele frequency changes in 28 loci, out of 82, could not be explained by genetic drift, thus it should be attributed to selection. The within-population expected herezogosity decreased, while the inter-population component of genetic variation increased (Labate, Lamkey and Woodman, 1999). Also, another study was conducted to investigate the genetic variation of progenitor inbred lines used to synthesize populations BSSS and BSCB1, as well as elite inbred lines derived from advanced cycles of selection (Hagdorn *et al*., 2003). A larger genetic distance was found between the BSSS and BSCB1 groups of lines derived from advanced cycles than between the group of lines derived from cycle 0. Thus, these studies confirm the success of RRS in increasing the genetic diversity of the two populations, which was one of the objectives of the method. Also, these results reinforce the hypothesis of the complementary effects of heterozygous genes in the population cross, and the hybrids between elite inbred lines derived from the two reciprocal populations.

2.3.3 Long-term recurrent selection experiments

Mass selection experiment for ear length in maize

A mass selection programme started at Iowa State University in 1963 to select for divergent ear length (i.e. short and long ears) in the maize population BSLE (López-Reynoso and Hallauer, 1998). A modified scheme of mass selection proposed by Gardner (1961), called grid selection, was used in this experiment. The scheme subdivides the selection field into 100 plots, each with 40 plants, among which three plants per plot (i.e. 7.5 percent selection

pressure) were selected for long and short ear. Each selection cycle was completed in one year. The linear selection response was 1.4 percent per cycle for longer ears, and 1.9 percent per cycle for shorter ears over 27 selection cycles. The rate of the inbreeding depression in selfed populations relative to unselfed was about 18 percent for longer ears and remained constant during the process of selection, whereas this rate gradually reduced for shorter ears as generations advanced, from 18.2 percent in the original population up to –2.9 percent in the 24th cycle (López-Reynoso and Hallauer, 1998). If the genetic effects of some loci are assumed to be dominant for longer ears, then the recessive alleles of these loci would be more easily fixed for shorter ears in the course of selection. Thus, no alleles with dominant effects would be segregating in the selected population for short ears after cycle 24, which would explain the absence of inbreeding depression in the short-ear population in comparison with the long-ear population. Genetic variance for ear length still remained in both populations after selection cycle 24, although the heritability of the trait was reduced.

Mass selection experiment for prolificacy in maize

A mass selection programme was initiated at the University of Wisconsin-Madison in 1971 to select for prolificacy (i.e. increase in number of ears per plant) in the maize open-pollinated population GG(MP) (de Leon and Coors, 2002). The selection response of the programme was an increase of 0.14 and 0.03 ears per plant per cycle at low and high plant densities (15 000 and 73 000 plants/ha), respectively, during 24 selection cycles. Most of the response was achieved between cycles 18 and 24. Indirect responses were also sensitive to selection. An increase in number of ears per plant brought a decrease in other ear traits affecting yield, such as ear length, ear diameter and kernel size, revealing that grain yield is a very complex trait and individual selection for one of the yield component does not necessarily have a significant effect on the yield trait.

Over a century of an Illinois long-term selection experiment for oil and protein content in the maize kernel

The Illinois long-term selection experiment begun in 1896, analysing percentage oil and protein in 163 ears of the maize open-pollinated variety 'Burr's White' (Dudley, 1977). The 24 ears highest in protein, the 12 ears lowest in protein, the 24 ears highest in oil, and the 12 ears lowest in oil were selected to initiate the Illinois high protein (IHP), Illinois low protein (ILP), Illinois high oil (IHO) and Illinois low oil (ILO) strains, respectively (Dudley and Lambert, 1992). Results and studies of this experiment have been published in many reports (Leng, 1962; Dudley and Lambert, 1969; Dudley, Lambert and Alexander, 1974; Moreno-González, Dudley and Lambert, 1975; Dudley, 1977; Dudley, Lambert and de la Roche, 1977; Dudley and Lambert, 1992; Dudley, 1994; Sughroue and Rochefort, 1994; Moose, Dudley and Rochefort, 2004; Laurie *et al*., 2004; Dudley and Lambert, 2004; Clark *et al*., 2006; Dudley, 2007, 2008). So far, 106 cycles of recurrent selection have been carried out on these strains (Dudley, 2007). The schemes of the selection methods were slightly modified through generations. Mass selection was used during generations 0–9; ear-to row during generations 10–25; and half-sib selection for percent oil and mass selection for percent protein during generations 26 to date (Dudley, 1977; Dudley and Lambert, 1992).

This experiment reveals the power of long-term recurrent selection for achieving progressively significant genetic changes in quantitative traits, such as protein and kernel oil content, through more than one hundred selection generations. Responses to selection for the high and low strains of oil and protein in the maize kernel were very important in the long run. Populations changed from 4.6 percent oil to 19.6 percent in IHO, and to 0.5 percent in ILO after 87 generations. At generation 89, selection for ILO was discontinued because a biological limit had been reached in terms of maintaining seed viability of the ILO strain (Dudley, Lambert and Alexander, 1974; Dudley and Lambert, 1992). For protein, populations changed from 10.9 percent to 32.5 percent in IHP and to 4.2 percent in ILP after 90 generations. Progress in the ILO and ILP lines had ceased in the latter generations (Dudley, Lambert and Alexander, 1974; Dudley and Lambert, 1992). In terms of additive genetic standard deviations (σ_a) of the populations, the genetic gains became huge, being 22 σ_a for IHO and 26 σ_a for IHP (Dudley and Lambert, 1992). These deviations are placed in the extreme tail of the normal distributions, with probabilities less than 10^{-105} and 10^{-145} for 22 σ_a and 26 σ_a, respectively. Thus, assuming that recombination of favourable loci could be gathered at random in a unique genotype in one generation, it would be necessary to grow an unimaginable number of maize plants (10^{105} and 10^{145} plants) to find one plant with the same oil and protein percentages as IHO and IHP, respectively. If no more than 10^{14} maize plants are currently grown in the world every year, it would be necessary to wait for at least 10^{91} and 10^{131} years to find at random the IHO and IHP strains that have been selected in only 100 years by applying simple recurrent selection methods. Genetic variance still remains in the IHO and IHP strains, because selection progress continued at the same rate in the two strains from generations 76 to 90. In addition, significant genetic variances were directly estimated in the strains in the 65th generation (Dudley and Lambert, 1969).

Besides the potential breeding benefit of developing maize strains for high and low oil and protein, this experiment has been a good tool to look into the intricate complexity of the genetic architecture of a quantitative trait. The experiment has also been a test bench to check quantitative theory and to estimate some genetic parameters otherwise difficult to compute. The question that arises is: What we can learn from this long-term selection experiment?

1. Recurrent selection was able to achieve a steady increase in the selection traits over more than one hundred generations. Mild selection (20 percent selection pressure) and adequate effective population size (>20) should have had a favourable effect in achieving this selection progress. The experiment has been a text-book example of selection. Recombination of selected genotypes created new genotypes distributed around new displaced means generation after generation. Thus, recombination and selection were the key points for reaching this huge genetic gain (i.e. 22 σ_a and 26 σ_a), which otherwise would not be possible to attain.
2. The genetic variance is not still exhausted. Reverse selection strains (i.e. RHO, RLO, RHP, and RLP) and switch-back selection from the reverse strain (SHO) clearly indicate that loci are still segregating in the populations and selection can be conducted in the desired direction at the same or higher rate as before.

3. By applying quantitative genetics theory to the genetic gains of the divergent selection strains, Dudley (1977) was able to estimate 54 loci for oil and 122 for protein, as well as the average allele frequency of alleles controlling the traits in the initial populations. Considering that oil and protein traits are not as complex as the yield trait in most of crop species, the number of loci responsible for yield should probably be higher.
4. Linkage disequilibrium in the coupling phase was detected for oil (Moreno-González, Dudley and Lambert, 1975) and protein (Dudley, 1994) using a Design III (DIII) of North Carolina in the F_2 and F_6 generations. This DIII was also able to estimate significant additive and dominance variances in the cross population. Reduction of additive variances from the F_2 to the F_6 generation suggested that loci for low and high oil and protein were coupled. For oil, there was no reduction in the dominance variance from the F_2 to the F_6 generation, suggesting that loci with dominance for low oil are combined with loci having dominance for high oil.
5. Single nucleotide polymorphism (SNP) molecular markers were used in the cross population of IHO × ILO, followed by 10 generations of random mating and one of selfing to look for associated QTLs (Laurie *et al.*, 2004; Clark *et al.*, 2006; Dudley, 2007). Molecular marker results confirmed the estimates of genetic parameters and the hypothesis proposed on the basis of quantitative genetics theory several years before. The number of QTLs estimated for oil was about 50, which was similar to the number of loci estimated by Dudley (1977). QTLs had small additive and dominance effects, which is congruent with the steady and continuous progress throughout generations and with no apparent exhaustion of genetic variance in the selected strains. Most of the QTLs with additive effects were in coupling phase, but some of them were also in repulsion, in agreement with the DIII studies (Moreno-González, Dudley and Lambert, 1975; Dudley, 1994). Breaking up the repulsion phase linkage would further increase selection response. QTLs had dominance effects for both high and low oil, as suggested by Moreno-González, Dudley and Lambert (1975).

2.3.5 Conclusions of empirical response to selection

A large body of evidence has been accumulated through selection experiments indicating that quantitative genetics is a useful empirical tool to model responses to selection. The genetic architecture of complex traits conforms to the infinitesimal hypothesis that postulates many genes segregating in the populations, with small additive and dominance effects and different allele frequencies. Also a few genes may have larger effects, and dominance effects may be present for both favourable and unfavourable alleles. Recurrent selection has been, is, and will be in the future, an effective strategy for improving complex traits in the desired direction. Choice of the selection method depends on the prevalence of additive or dominance effects. Intra-population selection methods mainly accumulate additive effects, while inter-population methods favour the presence of heterozygosity, differences in allele frequencies and the presence of dominance effects in the population cross. Genetic variance generally was not depleted in the selected populations, even in long-term

selection experiments. Likewise, heritability estimates were frequently no lower in the most advanced selected populations. Either the favourable alleles are hard to fix, or mutant alleles with small effects may naturally arise in the populations and subsequently may be captured during the selection process. Small effective population size is not an impediment for short-term selection, but it might limit selection in the long term. Inbreeding depression of selected population may be caused by small population size during the process of selection in traits where dominant favourable alleles are important. The inbreeding depression disappears when the selected populations are crossed to others.

Epistasis is well documented and has been recognized as a not rare phenomenon in Mendelian qualitative traits that are controlled by two or three loci. Thus, epistasis should be also expected in more complex traits. Analysis of generation means has been frequently used to look for epistasis in quantitative traits. The magnitude of epistasis effects detected was small relative to the additive and dominance effects in most of the studies, although some traits and elite crosses showed up significant epistasis (Moreno-González and Dudley, 1981; Melchinger, 1987; Melchinger, Schmidt and Geiger, 1988; Lamkey, Schnicker and Melchinger, 1995; Hinze and Lamkey, 2003). Either the favourable and unfavourable epistatic effects involving the trait cancel out through the genome, or the genetic models or the statistical methods used are not powerful enough to separate epistasis from other effects. Dudley (2008) analyzed 500 S_2 lines derived from the crosses IHO × ILO and IHP × ILP, using SNP molecular markers. He reported that epistasis could contribute to the long continued response to selection in the Illinois long-term selection strains, and also may help explain the continued success of commercial corn breeding.

2.4 SUMMARY AND CONCLUSIONS

We have described the evolution of selection and plant breeding theory, the conceptual basis of key components of plant breeding approaches, and empirical examples of selection. The contribution of plant breeding to the improvement of crop production and quality has been enormous. Critical in this contribution has been the implementation of more efficient breeding approaches supported by developments in quantitative genetics. Plant breeding has evolved and will continue to evolve, adopting classical and modern technologies (e.g. family selection, recurrent selection, multi-location evaluation, off-season nurseries, biotechnology) to increase efficiency of selection, to adjust to new environmental conditions and variable demands for crop utilization, and to implement sustainable production systems. Plant breeders and geneticists will continue the search for and adoption of the more effective methods to develop, identify and evaluate cultivars that can contribute to superior agronomic performance and sustainable profit.

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CHAPTER 3

Main stages of a plant breeding programme

Salvatore Ceccarelli

3.1 INTRODUCTION

This Chapter describes the main stages of a plant breeding programme, which represent the technical aspects of the process through which new varieties are generated. The organizational aspects will be dealt with in Chapter 9.

Schnell (1982) described three main stages of a plant breeding programme, namely:

1. ***Generating genetic variability.*** This includes:
   - making crosses (selection of parents, crossing techniques, type of crosses);
   - induced mutation; and
   - introduction of germplasm.
2. ***Selection,*** i.e. utilization of the genetic variability created in the first stage. This includes primarily the implementation of various breeding methods, such as classical pedigree, bulk-pedigree, backcross, hybrids, recurrent selection, F_2 progeny method (in self-pollinated crops), synthetic varieties and hybrids (in cross-pollinated crops), and clones and segregating populations (in vegetatively propagated crops).
3. ***Testing of experimental cultivars.*** This includes comparison between existing cultivars and the breeding materials emerging from stage 2, and the appropriate methodologies to conduct such comparisons.

There are two other important stages in a breeding programme: setting priorities; and dissemination of cultivars. These two steps are considered in Chapters 4 and 21, respectively.

Before describing the essential features of the various stages, it is important to underline that plant breeding is a cyclic process in which each step feeds information into the subsequent step, and each cycle feeds information into the next cycle. Therefore a major challenge in a breeding programme is how to capture the information that is generated, in a way that is sufficiently transparent for others (scientists and non-professionals) to use.

In conventional breeding programmes, most of this information represents the 'cumulative experience' or the 'knowledge of the germplasm' that the breeder slowly accumulates over the years. In a participatory programme, it is important to maintain the typical cyclic character of plant breeding to ensure that all the participants have the possibility of accumulating and sharing the information generated in each step and in each cycle.

3.2 GENERATING GENETIC VARIABILITY

3.2.1 Crosses

The most common way of generating variability across crops with different mating systems is to make artificial and deliberate crosses among parents selected for specific traits (see also Section 6.3 in Chapter 6) with the objective of combining them in at least a fraction of the progenies. Suitable parents can be received from other breeding programmes, or are extracted from germplasm collections after searching the passport data for specific attributes, or are successful cultivars, including the most commonly grown cultivars or landraces. Wild relatives (mostly in the case of rice, wheat and barley) are also used, although not by all breeding programmes and not routinely, as sources of genetic variation not available in the corresponding crop. The generalized use of wild relatives in breeding programmes is restricted in several cases by crossing barriers with the corresponding cultivated crop.

Because of the cyclic nature of a breeding programme, the majority of parents in any given cycle are represented by the best lines selected from the previous cycle.

Parental material for specific traits can also be identified through specific screening activities. A typical example is resistance to biotic stresses, for which a routine parallel activity in a breeding programme is to screen germplasm either under artificial inoculation or in 'hot spots', i.e. in a location with a very high incidence of natural infection or pest challenge. Another example is the case of quality characteristics, in which germplasm is screened for the presence or amount, or both, of specific compounds associated with a given food, feed or processing quality.

Parental material can also be characterized for the adaptation to different environments or countries, and therefore crosses can be made with the objective of producing breeding material with general adaptation to particular environments or countries. Examples are screening under controlled conditions for resistance to salinity, cold, drought, or for micro-elements such as boron, manganese or aluminium (Marshall, 1991; Karakousis *et al*., 2003).

Backcrossing—even though it is often described as a breeding method—actually refers to a type of cross to generate variability in a fashion that increases the frequency of desirable combination of traits, i.e. most of the traits from the recurrent parent and one or a few from the non-recurrent parent.

A number of molecular tools are available today to assist the breeder in the selection of parents and in subsequent stages, and they are described in Section 19.5 (Chapter 19).

The number of crosses made by a breeding programme depends on various factors, such as the number of different objectives, the resources available and the breeding method used, which affects the number of breeding lines generated. In general, a breeding programme with a regional perspective (i.e. serving a single country or state) performs between 150 and 200 crosses per season on average. The number can go up to several thousand in international breeding programmes. On the issue of the number of parents, Witcombe and Virk (2001) have proposed the use of a low number of crosses (see also Section 6.5.2 (Chapter 6) for further details on their theory).

When the objective of the breeding programme is to produce hybrids, the variability is generated through methods of population improvement based on recurrent selection or through crosses between elite inbred lines (see Section 11.5 in Chapter 11 for further details).

In a number of cross-pollinated species, hybrid vigour is exploited by developing synthetic varieties through intercrossing a number of genotypes of known superior combining ability, i.e. genotypes that are known to give superior hybrid performance when crossed in all combinations. In contrast to hybrids, the seed of synthetic varieties can be used for succeeding seasons, and for this reason synthetic varieties are common in crops, such as forage crops, where cost precludes the development or use of hybrid varieties.

3.2.2 Exploiting existing variability

It is widely recognized that landraces and wild relatives harbour large amounts of genetic variability, which, in the case of self-pollinated crops, is readily available, as landraces and wild relatives are largely composed of different homozygotes. In these cases, the first step is represented by the collection of single spikes or plants, while their evaluation represents the second stage of the breeding programme.

3.2.3 Mutation

The use of artificially induced mutations, described in detail in Chapter 8, dates back to the origin of plant breeding as one of the ways to generate genetic variability beyond and besides that available naturally. The reader is referred to that Chapter for the technical aspects of induced mutations in plant breeding.

3.2.4 Generating genetic variability in a participatory breeding programme

In a truly participatory breeding programme, i.e. a programme that maintains the cyclic nature of a breeding programme, it is not extremely important who generates variability by, for example, making crosses, in order to categorize the type of participation. In fact, in any breeding programme, the degree of participation is determined by who selects the parental material. In a participatory breeding programme, if the farmers participate in the selection, they implicitly participate in the choice of the parents even if they do not physically make the crosses.

In a participatory programme, farmers can also contribute to the first step by suggesting the type of germplasm that is more likely to be acceptable in a given area.

3.3 SELECTION

The main characteristic of the selection stage is the utilization and narrowing down of the variability generated in the first stage and includes various steps by which the large diversity of breeding material (genetically of different types, depending on the mating system of the crop) is reduced to a number of lines suitable for the third stage.

The second stage is critical because the choices that are made during the various steps depend on the genetic control of the trait(s) under selection and on the environment in which the decisions are taken. Selection environments should be chosen to jointly reproduce the response of materials over the target region, or to be representative of the target environments of different agro-ecological subregions when breeding distinct varieties for each subregion (see Chapters 9 and 20).

The methods on how to handle the breeding material during the second stage vary considerably depending on the mating system of the crop, and these are dealt with in the respective chapters.

3.3.1 Self-pollinated crops

In self-pollinated crops, the products of the first stage are known as 'segregating populations', and the most common methods of handling them are described in Chapter 10. All the methods have in common a progressive increase in homozygosity, a reduction in the genetic variance within families, and an increase in the genetic variance between families.

In conventional breeding programmes, all the steps of stage 2 take place typically within a research station. In the initial steps of this stage the amount of breeding material is very large (often several thousand entries), and it is hard to organize their evaluation outside a research station. However, some breeding methods are particularly suited to the evaluation of the early segregating populations in the target environment (see Chapters 9 and 22), and enable the participation of farmers already at such an early stage. For example, in the bulk-pedigree method, the segregating populations, usually F_2 or F_3, each derived from a different cross, can be tested in a number of locations using an experimental layout similar to the yield trials (see stage 3), with the number of locations dependent on the diversity of the target population of environments and on the amount of seed available. In such cases, the

selection can be practised between crosses in the target environment, and within crosses on station for traits with high heritability.

Single-seed descent (described in Chapter 10) is one of the best ways of exploiting the variability within a superior cross and is fully compatible with a participatory programme as long as farmers contribute to the identification of the superior cross.

3.3.2 Cross-pollinated crops

The methods of exploiting the variability generated in the first stage in cross-pollinated crops vary according to the final product that the programme aims to produce (a hybrid, an open-pollinated variety, a synthetic variety, etc.).

When the objective of the breeding programme is to produce hybrids, the variability generated through methods of population improvement is exploited through the development of inbred lines (the methods to do that are described in Chapter 11), which are then crossed to produce the commercial hybrids. In the case of hybrid production, farmers could be involved both in the second step by contributing to the evaluation of the inbred lines, and in the third step by participating in the evaluation of the hybrids.

3.4 TESTING OF EXPERIMENTAL CULTIVARS

Testing of potential cultivars is the last stage of a breeding programme, which eventually ends with a new variety recommended for cultivation.

Usually, this stage takes place partly on research stations and partly in farmers' fields. However, there are exceptions, the best known being the breeding programmes in Australia where all the yield testing actually takes place in farmers' fields.

In most of the cases where the yield testing is conducted partly on station and partly in farmers' fields, the testing on research stations usually covers a period of three years. Most commonly, the number of breeding lines entering the testing stage is progressively reduced by discarding those that performed below a given standard. The most commonly used agronomic trait used to promote or discard breeding material during the testing stage of a breeding programme is grain yield. However, when the objectives of a breeding programme include, for example, quality characteristics and disease resistance, traits such as seed size or reaction to diseases complement grain yield.

The testing of experimental cultivars has a number of methodological and philosophical issues. The methodological issues include field plot techniques, design of trials (replicated vs. unreplicated trials), analysis of variety trials, and the organization and structure of Multi-Environment Trials (METs). The philosophical issue is primarily whether the testing of experimental cultivars should be conducted in an optimum climatic and agronomic environment or should be conducted in the target environment. When selection is conducted in the target environment, the breeding programme has to decide how many and which target environments to serve (Chapter 20).

One of the basic principles to apply in implementing the third stage of a plant breeding programme is that *field trials are expensive, and therefore the breeder should always find an optimal compromise between the number and the size of the trials, their precision, and the amount and the relevance of the information generated.* An ideal system of testing of experimental cultivars is a system that has the capacity to self-monitor its efficiency, effectiveness and relevance, and has flexibility to allow changes.

3.5 EFFICIENT EXPERIMENTAL PROCEDURES

Field plot techniques, as well as the choice of efficient experimental designs, aim at reducing the experimental error, thereby increasing the heritability and increasing the response to selection. Adopting efficient experimental procedures has paramount importance not only in the selection stage, but also in the testing stage of a breeding programme.

3.5.1 Field plot techniques

It is commonly believed that error variances tend to be larger under stress than under non-stress conditions, and this belief is a common justification for breeders to do most of the work on station or under optimum conditions. Even though this belief is hardly supported by experimental evidence (Al Yassin *et al*., 2005; Comadran *et al*., 2008), and because at the moment of planting it is difficult to predict how uniform a particular piece of land is going to be, it is always safer to put in place a set of measures to control at least some predictable sources of experimental error.

When genotypes are compared at increasing levels of moisture stress, small variations in soil depth and texture have increasingly large effects on plot-to-plot variability. Under these conditions, competition among genotypes for water also increases and bordering becomes critical (Fischer, 1981). For example, the yield of the outside row as a percentage of the yield of well-bordered rows in a maize nursery increased from 124 percent to 185 percent as yield levels were reduced by drought from 5 to 1 t/ha (Edmeades, pers. comm.). Many breeding programmes assume that all plots are equally affected by the border effect, and do not remove plants bordering alleys prior to harvest. However, this can introduce significant error in yield estimates, as there is strong genotype × border effect interaction. In fact, the decline in plant height from the edge to the centre of the plot can be used when selecting stress-tolerant lines (Rosenow, 1987; Blum, 1988). Reduction of border effects can be achieved very effectively in small-grain cereals by avoiding empty rows between adjacent plots and by planting the alleys. The resulting 'dirty' alleys are not very attractive, but their effect on uniformity within the plots is remarkable. Removing the alleys can be done at heading or shortly before maturity. This technique is particularly useful when testing is done in farmers' fields because, as mentioned in Chapter 9, farmers do not like to leave land empty on their property.

The control of border effects is also important in breeding nurseries, usually planted as individual rows or as two-row plots. The common practice is to leave one empty row between adjacent entries. The result is that everything we observe is border effect—with the exception, perhaps, of simply inherited characters.

Small plots should be avoided as much as possible when conducting yield trials. Table 3.1 shows an example of the effect of plot size on selection efficiency in barley on a dry site.

Competition among progenies grown in single- or double-row plots may lead to the identification of genotypes that owe their yield superiority only to the lack of aggressive rooting or smaller plant height of their neighbours. These advantages are nullified when the selected 'superior' cultivar is grown in pure stand. Constraints on seed per progeny and cost of labour and land often make additional bordering difficult.

Missing rows also have a marked effect on the performance of neighbouring rows, and it is advisable to check lines for germi-

TABLE 3.1
Efficiency of selection for grain yield as affected by plot size during the testing stage

Selection criterion in the previous season	No. of lines	Plot size	No. and percentage of lines outyielding the best local check	
			Dry site	Wet site
Grain yield	13	2 rows	5 (38.5%)	1 (7.7%)
Grain yield	21	4 rows	17 (81.0%)	2 (9.5%)

TABLE 3.2
Average yield (kg/ha) and coefficient of variation in barley yield trials conducted in two locations in northern Syrian Arab Republic as lattice designs

Trial No.	Tel Hadya (352.6 mm rainfall)		Bouider (243.6 mm rainfall)	
	Mean	c.v.	Mean	c.v
1	3715	17.7	1006	11.6
2	3337	12.8	777	15.8
3	3290	10.2	808	12.3
4	3005	15.3	955	12.6
5	2759	15.1	923	14.9
6	3195	12.1	1025	14.1
7	2993	13.7	1029	8.2
8	3156	15.0	997	13.1
9	3271	15.4	1031	11.0

nation prior to establishing a trial on a dry site if there is reason to believe that some entries will not germinate completely.

A series of check entries, spaced at regular intervals throughout unreplicated progeny trials, is essential to compensate for the effects of soil variability. Plot data are expressed relative to the performance of the check, adjusted for the physical distance between the nearest check plots and the plot in question. The check genotypes must always include the farmers' cultivar(s), lines that are well known to the breeder, and the best lines previously identified by the breeding programme. A progeny trial should be arranged in the field to ensure that check entries will not all be in the same columns; rather, they should form a grid which will provide a visual impression of the uniformity (or the variability) of the field.

When all these techniques are used on sites with low yield potential due, for example, to moisture stress, environmental variability can be kept at levels comparable with those of well-managed research stations with high average yields. As an example of what could be achieved by the package of plot techniques described in this section and the use of the experimental designs described in the next section in controlling environmental variability, we compared the coefficient of variation of nine trials grown on a stress site (average yield = 0.95 t/ha) and on a non-stress site (average yield = 3.19 t/ha) (Table 3.2). At yield levels where breeders usually do not work because the coefficient of variation is too large, the lattice design was capable, in most of the trials, of keeping it within acceptable limits.

A common source of error in conducting yield trials is an uneven plot length resulting from trimming plots to eliminate the edge effect. An effective way of ensuring a uniform plot length is to spray a herbicide using booms placed on a rigid arm

at a distance equivalent to the desired plot length.

3.5.2 Design of trials

The choice of an appropriate experimental design is another important decision affecting the precision of a trial.

One of the first issues in discussing the design of a trial is the alternative of replicated vs. unreplicated trials. In the testing stage of a breeding programme, which typically goes through three steps, the number of breeding lines being tested is usually in the range of several hundreds or even a few thousand in the first step, reducing to usually less than 50 in the third.

The use of replicated designs with several hundred or a few thousand lines implies the use of large areas, which makes the control of experimental error more problematic. In this step the number of experimental units (plots) per breeding line is limited by the amount of seed, which is usually small at this stage.

The combination of limited amounts of seed and of a large number of lines has made popular the use of unreplicated designs with systematic checks and with a row and column arrangement of the plot, which makes it possible to use spatial analysis (Singh *et al*., 2003).

One improvement over the unreplicated design with systematic checks is a partially replicated design where only a certain percentage of entries is replicated while still maintaining the systematic (also called grid) checks. In addition to the expected higher precision, the design responds to a frequent problem in the initial stages of testing, that is the different amount of seed available for different entries.

In replicated yield trials, where genotypes under test may number 200–500, improved statistical designs can lead to important increases in trial efficiency.

Unfortunately, despite the greater efficiency of lattice designs, of generalized lattices (Patterson and Hunter, 1983) and of neighbour analysis (Cullis and Gleeson, 1989), the randomized complete block design is still dominant in many breeding programmes in developing countries, particularly in those conditions where an increase in trial efficiency is most needed.

The use of generalized lattice designs (Patterson and Williams, 1976) combines good error control with flexibility in the numbers of treatments required. A promising extension of this design that removes both row and column effects has been described by Patterson and Robinson (1989). Nearest-neighbour analysis (e.g. Wilkinson *et al*., 1983; Hinz, 1987) have been used extensively in Australia to remove the effects of gradients of moisture stress within replicated and unreplicated trials (Marshall, 1987).

Spatial variability is a reality in field trials and a proportion of this is accounted for as inter-block variability by using block (complete or incomplete) designs. However, a large amount of spatial variability still remains unaccounted for, and this may lead to erroneous conclusions. To further capture this unaccounted-for variation (which is mainly due to intra-block variation), yield data from variety yield trials can be analysed using various spatial models. Singh *et al*. (2003) showed that spatial models add considerable value to trials; the 'best' spatial models gave efficiency values of over 330 percent in winter-sown chickpea, 140 percent in lentil and 150 percent in barley trials. Furthermore, the use of these best models resulted in a change in the ranking of genotypes (on the basis of mean yield), which therefore resulted in a different set of genotypes being selected for high yield. It is recommended that

(1) incomplete block designs be used in variety trials; (2) the Akaike Information Criterion (developed by Akaike under the name of "An Information Criterion" (AIC) in 1971 and proposed in Akaike (1974) as a measure of the goodness of fit of an estimated statistical model) be used to select the best spatial model; and (3) genotypes be selected after the use of this model. The selected model would most effectively account for spatial variability in the field trials, improve selection of the most desirable genotypes, and therefore improve the efficiency of breeding programmes.

Additional information on this issue, also with regard to useful software, is given in Chapter 20.

3.6 MULTI-ENVIRONMENT TRIALS

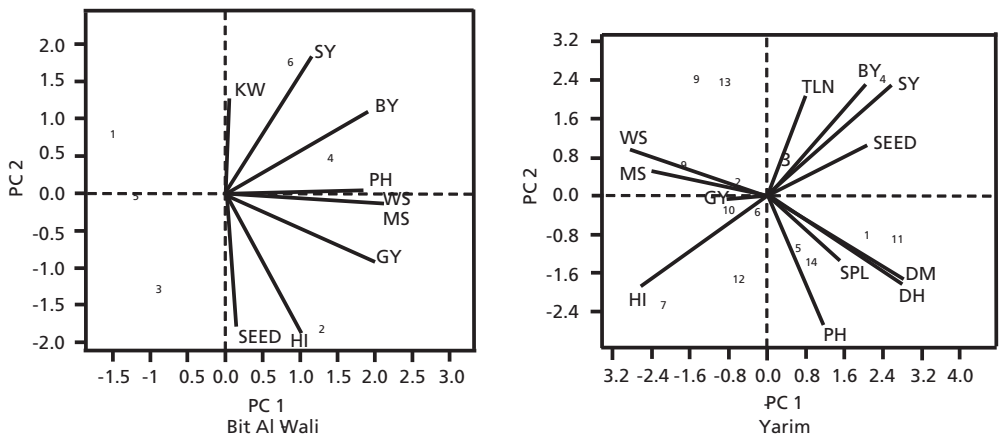
The last stage of a plant breeding programme aims also at understanding the reaction of the breeding material to a multitude of environments, i.e. locations, years, and possibly different types of agronomic management. During this process, the amount of breeding material is progressively reduced, and the number of locations and the number of replications per location is progressively increased. In the first level of yield testing the general tendency is to have as many locations as possible (the main limiting factor being the amount of seed available), sacrificing number of replications per location. Because of the limited precision of these trials, most breeders will mostly do negative selection, i.e. discarding the obviously inferior breeding material. As the material is advanced, the precision of the trials also increases. At the end of the testing phase, and for the surviving breeding material, data are available from a number of locations and years, which can be analysed with one of the techniques described in Chapter 20.

When METs are conducted in farmers fields, which is common in some conventional breeding programmes (in Australia, for example), the breeder may face organizational issues different from those in a research station (Chapter 9). METs planted in farmers fields are not yet participatory plant breeding because, as is the case for the Australian breeding programmes, farmers only make land available against the payment of a rent and do not participate in decisions related to the selection of breeding material.

One important aspect of a MET is the ability to subdivide Genotype × Environment interaction into Genotype × Location (G×L) and Genotype × Year (G×Y) interactions. Distinguishing these interactions is important because the two differ in importance with regard to plant breeding. While G×Y interactions are largely unpredictable, G×L interactions can be predicted. In the case of G×L interaction, therefore, it is essential to assess both its magnitude (relative to G) and its repeatability over time. Such an assessment allows the target population of environments to be divided into subsets in a way that ensures that there is a high degree of repeatability or consistency (Kempthorne, 1952) of G×L interaction between subsets and a low degree of repeatability within subsets. Eventually this leads to the identification of high-yielding, stable genotypes adapted either specifically or widely, and to clusters of response-similar environments, which can contribute to defining a selection strategy and to locating a small number of optimal selection sites for future breeding. These topics are extensively discussed in Chapter 20.

The use of biplots as means to graphically display genotype adaptation patterns and environment similarity for GE interaction effects is discussed in Chapter 20.

FIGURE 3.1
Biplots of farmers' preferences in barley grown in two villages in Yemen (6 and 14 varieties, respectively)



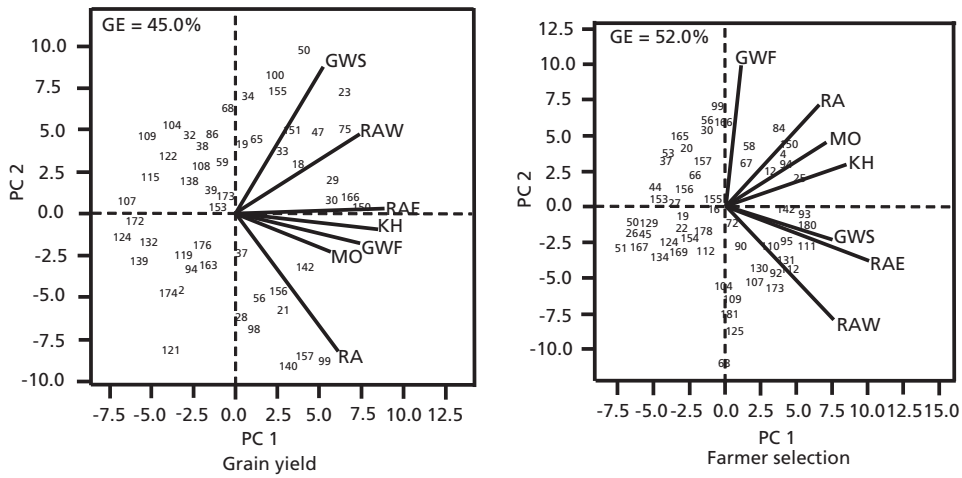
Key: PH = plant height; KW = 1000-kernel weight; SEED = number of kernels/spike; BY, GY and SY = biological, grain and straw yields, respectively; HI = harvest index; DH = days to heading; DM = days to maturity; TLN = tiller number; MS = male selection; WS = female selection.

Biplots, such as those envisaged by Yan and Tinker (2005), can also be useful in PPB programmes to assess (1) the traits used by farmers as selection criteria; (2) the consistency of selection criteria across environments; and (3) whether locations that show repeatable G × Y interactions and that therefore could be lumped together as one subregion (or mega-environment), actually differ in farmer preferences.

An example of using the biplot to assess which traits are actually used by farmers as selection criteria and the consistency of selection across environments in given in Figure 3.1. A narrow angle between the vectors for farmer selection (MS for the males and WS for females) and the vector for a plant character indicates a strong preference for that given character. This is the case of plant height in Bit Al Wali, where both men and women selected for tall plants, and to a slightly less extent for grain yield. It can be noted that in the case of the second village, the preferences were reversed, the wide angle between the vectors for farmer selection and those for plant height suggesting that farmers in Yarim selected for short plants and for early heading and maturity, as indicated by the direction of the vectors of these two characters opposite to the vectors for farmers' selection.

Figure 3.2 shows an example of differences in locations clustering depending on whether grain yield or farmers' preferences are used. On the basis of grain yield, four locations, namely MO, GWF, KH and RAE, are closely correlated; they are likely to represent a similar environment as they discriminate similarly among genotypes. If this is repeatable over time, then it can be argued that any one of the four will be sufficient to represent that given macro-environment, thus leading to a considerable saving in resources. However, only 2 of the 4 locations (MO and KH) are closely correlated also for farmer preference, while GWF and RAE are independent from each other and weakly correlated with the pre-

FIGURE 3.2
Biplots of grain yield and farmers' preferences for 182 barley genotypes grown in six farmers' fields and one research station in Jordan (not all genotypes are shown)



GWF = Gweer farmer field; GWS = Gweer research station; RA = Rabba; KH = Khanasri; MO = Mohay; RAE = Ramtha East; and RAW = Ramtha West.

vious two. Therefore, if these patterns are repeatable over time, it would be advisable to only drop either MO or KH.

3.7 CONCLUSION

In a typical breeding programme it is possible to recognize three distinct stages: the creation of genetic variability; selection of the desirable gene combinations; and the final testing of these desirable gene combinations.

In a truly participatory breeding programme, farmers participate in all the three stages. When farmers participate in only the last stage (as often is the case), it is more appropriate to talk of participatory variety selection (PVS). There are important conceptual differences between the two. PVS is selection among (usually only a few) finished or nearly finished varieties, such as when farmers choose from on-farm variety trials, which are the very last stage of a breeding programme, and, very importantly, it is a linear process. In contrast, in PPB farmers participate in selection when genetic variability is at or near its maximum, such as selection between or within early segregating populations. Also, contrary to PVS, PPB is a cyclic process.

Ultimately, a participatory plant breeding programme can use and benefit from the use of the most advanced experimental designs and analytical tools.

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CHAPTER 4

Methodologies for priority setting

Eva Weltzien and Anja Christinck

4.1 WHY DO WE NEED METHODOLOGIES FOR PRIORITY SETTING IN A PLANT BREEDING PROGRAMME?

Productivity improvements have been the key objective of most plant breeding programmes to date: international, national, private and public alike. Other major breeding objectives are indirectly related to this goal: resistance to pests and diseases, for instance, or adaptation to abiotic stresses (such as drought or low soil fertility), and aim at increasing or stabilizing yield or to allow higher production under certain environmental conditions.

Another group of 'classical' breeding objectives focus on adding 'value' to crops by improving their qualities for industrial processing, their storability, or by meeting certain consumer preferences. Some breeding programmes concentrate on increasing the nutritional value of staple food crops, an approach that is also known as biofortification (HarvestPlus, 2007).

Increasing the yield of important food crops was seen as the answer for overcoming food shortages and reducing hunger in the world. In fact, the production volume per hectare of some major food crops has increased about threefold in the last 70 years, partly as a result of plant breeding and partly due to intensification of farm management (Becker, 1993). In recent years, however, evidence has been mounting that the global availability of staple food alone is not sufficient for reducing hunger and malnutrition. Food insecurity is closely related to poverty in general: even if food is available, many poor people, including poor farmers, lack access to it. The alleviation of poverty has therefore become a key development goal. It is at the top of the agenda for many development organizations, both governmental and non-governmental, and also for international agricultural research centres. In view of this goal, international breeding programmes and their national partners have been compelled to redefine their programme objectives and specific targets. Crop breeding programmes, for instance, must be re-oriented towards the needs of poor farmers and other specific user groups. However, user differentiation and gender considerations are new concepts for many breeding programmes; developing new and 'better' varieties was assumed to be a largely user-neutral technology.

Furthermore, the benefits from newly developed varieties are not evenly distributed; in some regions, for example sub-Saharan Africa, where poor soil fertility and erratic rainfalls limit the potential for agricultural production, there has practically been no yield increase in major food crops in the last 20–30 years (FAOSTAT data, 2006). In such regions, farmers have often developed complex farming systems and strategies for reducing environmental risks. However, social, political and economic change can weaken such systems, leading to instability and overexploitation of the natural resources. Plant breeding for such situations requires different approaches: approaches that are based on a deep understanding of the functions of crops within the entire system, including farming, nutrition, local knowledge and technologies. Setting priorities for such programmes needs to be forward looking, as it may take at least ten generations before new products become available. They then need to be adapted to farmers' needs and production systems. Simple strategies, such as improving yield by increasing the ratio of the edible part at the expense of other plant organs (i.e. foliage, roots), do not generally work under such conditions. For example, certain 'minor' characteristics may

BOX 4.1

The value of pearl millet straw in drought years

In western Rajasthan, drought occurs so regularly that farmers have developed their strategies to cope with it. Many farmers, even though interested in new varieties for testing and experimentation, grow traditional pearl millet landraces. In good years, the yield of the landraces is moderate, but their real value is revealed in severe drought years: even if the grain yield may be strongly reduced, they produce some grain as food and biomass for feeding the animals. Many modern varieties fail totally under such conditions, producing neither straw nor grain.

There are several possibilities for people to find grain for human nutrition: some may have stored a surplus from previous years, or one can do labour work and buy grain from other regions in the market. In severe situations, food aid may be distributed by governmental or private aid agencies. But starvation of animals hits a farmer family hard for years; the animals are an important source of income, besides providing dung, draught power, milk or wool for the family and the farm.

be re-lated to environmental adaptation, or non-edible plant parts may have a high value in particular situations (see Box 4.1).

Another point receiving increasing attention is the conservation of agricultural biodiversity. Many countries have signed the Convention on Biological Diversity (CBD), or the legally binding International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA). It is now widely recognized that industrialized farming has led to significant losses of biodiversity in agricultural systems. This is due to the use of only a few, widely adapted, varieties; the narrow genetic base of the breeding materials; and testing and release procedures allowing only the dissemination of a limited number of relatively uniform varieties. Economic considerations are one reason why previously selected breeding material is used as much as possible, but it is thus reducing the genetic diversity among the newly developed varieties. Landraces and wild plants are not incorporated as much as they could be, because it may take more time to derive stable and uniform varieties from such material, thus increasing the cost of such programmes (Haussmann *et al*., 2004). Furthermore, the business economics of breeding firms require a large geographical distribution of varieties within a few years, which conflicts with biodiversity considerations and other aspects of regional differentiation, such as respecting food culture and consumer preferences. Locally important crops often do not reach the scale of distribution that is needed by breeding institutions for them to invest in new varieties. However, as a result of international commitments, national and international breeding programmes are obliged to initiate efforts for broadening the genetic base of breeding materials, according to the Global Plan of Action (FAO, 1996), and the International Treaty for PGRFA (ITPGRFA, no date).

Decentralized breeding programmes, based on local crop germplasm and seed distribution systems, in contrast, could be an important step towards increasing the level of biodiversity in farmers' fields. Moreover, the goal of conserving agro-biodiversity could effectively be linked to efforts to increase food security and reduce poverty: Many 'minor' or traditional crops

(or crop varieties) have outstanding nutritional qualities, are well adapted to marginal conditions and low input farming, or open up possibilities for income generation (IPGRI/GFU/MSSRF, 2005).

Thus, breeding programmes today often have to be designed in a manner different from the past. To meet the above-mentioned new goals, they tend to be less centralized, more targeted towards specific user groups and often use different germplasm. However, this is not all. To obtain impacts beyond a very local scale, approaches have to be developed that address large geographical areas while at the same time respecting agro-ecological and socio-cultural differences. This usually requires cooperation among different organizations that work at different scales, and often have diverse agendas and backgrounds. Consequently, methodologies for priority setting have to be adapted for such cooperation to make the process transparent and acceptable for all stakeholders.

The management of social cooperation, learning and decision-making processes is, as such, new for most plant breeders and their institutions. However, experiences exist from other disciplines, particularly social and economic sciences; here one can build on fundamental expertise in the fields of knowledge systems, communication, social learning and management (Leeuwis, 2004; Manktelow, 2003).

4.2 PARTICIPATORY PLANT BREEDING

The concept of participatory plant breeding (PPB) emerged in the late 1980s as part of a general development in participatory research methodologies during that period. Increased user orientation and more efficient allocation of research funds; higher adoption rates; a close relation to local cultures, knowledge and skills; empowerment of farmers; and overcoming typical limitations of 'science' in the development context—all these factors are the potential advantages of participatory plant breeding (Ashby and Sperling, 1995; Weltzien *et al.*, 2003).

PPB includes all approaches to genetic plant improvement involving close farmer–researcher collaboration. The term particularly refers to active involvement of farmers in at least one of the stages of a plant breeding programme, including setting objectives, generating variability, selecting and testing, as well as seed production and distribution.

This active involvement of farmers can take different forms. Farmer participation can be consultative, if farmers are interviewed on agro-ecological issues, or on the performance of test varieties. More active forms of farmer participation include, for example, trial management, selection, priority setting and the development of action plans, or the overall management and implementation of the project (Farnworth and Jiggins, 2003; Lilja and Ashby, 1999). Which degree of farmer participation is appropriate and in which phase of a breeding programme depends largely on the goals of the programme, as well as the type of improvements needed, and it is thus also an issue for priority setting (see Section 4.5 below, under Roles and Responsibilities).

4.3 PRIORITY SETTING AS AN ITERATIVE PROCESS

Setting priorities is an important part of professional plant breeding work. Time and resources are usually limited, and they have to be allocated in a rational way in order to reach the goals of the breeding programme. Thus, considering issues and methodologies for priority setting is a necessary step for any plant breeding programme, irrespec-

tive of the degree of farmer participation or the institutional setting. However, little has been reported to date on methodologies for priority setting in plant breeding programmes. Resource allocation, primarily during the phase of testing experimental cultivars, has been researched intensely, usually based on models for maximizing genetic gain, thus focusing on one or two key traits (e.g. Cooper and Byth, 1996).

We regard priority setting as an iterative and progressive process that will be considered at many stages during a plant breeding programme, not only in the project planning phase. It is often not possible to anticipate all the options that may emerge in the course of the research process. Priority setting methodologies should therefore become part of the regular project work, in a way that allows adjustments and further development of goals and priorities as the project work evolves.

In the following sections we will look at issues for priority setting first, and then suggest methods and communication tools that could help to achieve a transparent process and productive outcomes.

4.4 ISSUES FOR PRIORITY SETTING

Clear priorities need to be set for a number of issues. The **goals** are the guiding principles for priority setting in any project of a defined duration, scale and scope. At the same time, the goals themselves are also an issue for priority setting, as complex, conflicting or too general goals are not likely to be reached through technical plant breeding work alone.

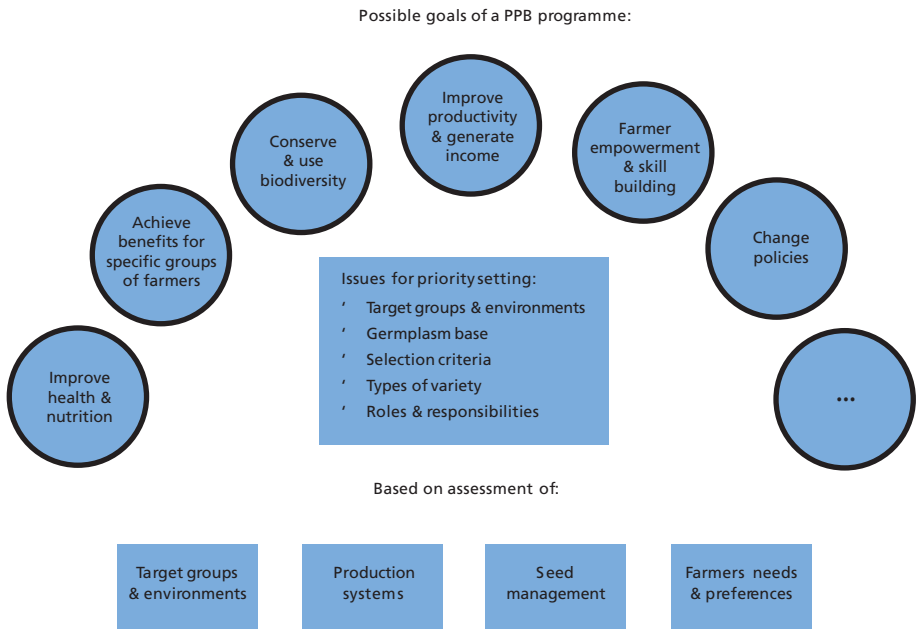
For plant breeding programmes, it is vital to define the **target group(s)** and **the target environment(s),** i.e. production conditions under which the newly identified varieties should perform better than existing cultivars, and the specific needs of the target group of farmers. Closely linked to this are priority traits to be used as **selection criteria.** To achieve good progress from selection, the **germplasm base** must be chosen appropriately, based on profound knowledge of the available options. It is also important to discuss what **type of variety** might be the most appropriate for achieving the project or programme goals. Part of this issue is also to address the question of intravarietal diversity: how much of it would be beneficial or necessary, and for which traits. An issue that is often left until activities are planned is the identification of key **roles and responsibilities of partners**. However, since different options for sharing responsibilities between partners have a major impact on some of the goals, it is important to consider them from the outset of the breeding programme. The following sections explain in more detail how the different issues for priority setting for a breeding programme relate to the overarching goals in specific situations.

4.4.1 Goals as a basis for priority setting

All breeding programmes have at least one goal related to improving production, such as yield, yield stability or a higher product value. Many PPBs have additional goals, such as the conservation of local diversity, skill building and empowerment of farmers, policy and regulatory changes, increasing research efficiency, or benefits to specific users. Many of these goals tend to be implicit and depend on the institutional background and on the 'history' of the breeding project.

Each organization and institution has their own implicit goals that are not always easily communicated. Thus close interaction, exchange visits, and joint planning workshops that are held variously in the

FIGURE 4.1
Issues for priority setting in a plant breeding programme in relation to the overall goals and based on the assessment of target groups and environments, production systems, seed management, and farmers needs and preferences



Source: Weltzien, 2005.

different partners' workplace (e.g. research station, village, trading place) are important to achieve a mutual understanding of the different partners' perspectives. It is also understood that the relative importance of the different goals may change as the project and, foremost, the partnership advances and evolves.

If the project work involves close interaction between farmers or farmer organizations and researchers, it is particularly important to clarify the goals from the project planning phase. For many farmers, it is not easy to understand what scientists do and how research is organized. As a consequence, they may be tempted to overestimate the direct effects of the research on yield or income generation, or they may even expect other benefits from the cooperation, which cannot be fulfilled by a breeding programme. Such general aims of the people could perhaps better be addressed by activities other than plant breeding, or by establishing partnerships with marketing organizations or food processing companies (and including their specific goals into the breeding programme).

From goals to priority setting

The goals have been described as the guiding principles for priority setting. At the same time, the priority setting process builds on understanding the present situation, anticipated changes, and farmers' needs. A detailed analysis of the production environment is required, including existing

varieties and how they are used by farmers, their preferences and relevant resources (i.e. local knowledge, skills, germplasm). In particular, it is necessary to identify the major constraints to production increases and income generation. Participatory methods for such situation analysis have been described in detail by Christinck, Weltzien and Hoffmann (2005). An open dialogue in the course of which all partners evaluate potential options and obstacles for future breeding activities could then follow (see Section 4.6 of this chapter). This approach is graphically summarized in Figure 4.1.

4.4.2 Target groups and target environments

Identifying the target environment and target group in view of the overall project goals is generally among the first strategic decisions to be taken in a plant breeding project. We therefore suggest a few subjects for consideration, which refer to agro-ecological as well as socio-economic factors.

Broad versus narrow adaptation, and the impact of PPB

The issue that certain plant types or varieties may perform differently in different environments is called 'genotype by environment interaction' by plant breeders. In general, most plant breeders tend to give preference to those populations that perform well under a wide range of conditions; this ability of plant populations is known as 'broad adaptation'.

Broadly adapted varieties are also the prime matter of interest for seed companies, as the potential profit from the entire release and multiplication 'business' is usually related to the scale of distribution. However, these varieties, if tested on research stations in multi-locational trials, may fail under the conditions of poor farmers working with limited resources and under marginal agro-climatic conditions. Ceccarelli, Grando and Booth (1996) and Ceccarelli *et al.* (2000) have shown theoretically and practically that interactions between genotype and environment can be positively exploited if the selection is done in the target environment, e.g. farmers' fields. Farmers as well as scientists successfully selected populations or experimental lines that produced better under the farmers' conditions than other varieties grown previously by those farmers. Experiences of other research groups, with various crops in differing natural and socio-cultural environments, support this understanding (Goyal, Joshi and Witcombe, 2001; Mekbib, 1997; Sperling, Loevinsohn and Ntabomvura, 1993; Weltzien *et al.*, 2003). Narrow adaptation to specific conditions, leading to the selection of many different cultivars for various conditions and purposes, is often regarded as an advantage of the PPB approach: it serves specific needs of farmers and enhances the level of agrobiodiversity in farmers' fields (Sperling, Loevinsohn and Ntabomvura, 1993; Joshi and Witcombe, 2001).

However, a possible criticism regarding decentralized plant breeding programmes could be that, due to the focus on specific, often marginal environments, and only the local importance of the varieties developed, their impact remains insignificant. Only a very few farmers who produce mainly for their own subsistence and modest requirements would profit from the activities, and this would never justify the breeding efforts, let alone the cost of official variety release and seed multiplication.

At the same time, there are also cases where varieties developed through PPB programmes are not necessarily so narrowly adapted. In Nepal, for example,

a rice variety selected by farmers in a high-altitude environment was adopted by many farmers in the mid-altitude regions as well (Joshi, Sthapit and Witcombe, 2001). Also, rice varieties developed in a participatory breeding programme in Nepal were superior to check varieties in a region of Bangladesh, where rainfed agriculture prevails (Witcombe *et al*., 2005). Obviously, much depends on the characteristics of the varieties, the conditions under which they were selected and the limitations that were addressed and overcome through the plant breeding activities. Thus, information on target regions and how representative these are for other farmers of a larger area will be of vast importance for the later impact of the project.

Identifying, specifying and delineating the target environments for a breeding programme more precisely is often done by analysing multi-location trials through which a broad range of potential varieties for a region can be evaluated. Calculating correlations between performance traits from the different testing sites usually gives an initial impression about the differences between the sites with respect to adaptation (Atlin, Paris and Courtois, 2002). If sufficient data is available, or can be generated during the course of the project, more complex statistical tools can be employed by breeders in order to delineate target environments and develop a selection and testing strategy for new varieties (e.g. Cooper *et al*., 1999; Cooper and Byth, 1996; Annicchiarico, Chapter 20). These statistical tools do not require farmer participation, but give a much more realistic assessment of the situation if the trials used for these analyses were conducted by farmers in farmers' fields, using farmers' selection and evaluation criteria. Similarly, farmers' description of requirements for adaptation to a specific zone can be a useful input, which could actually save efforts on long-term expensive experimentation and analysis (van Oosterom, Whitaker and Weltzien, 1996; van Oosterom *et al*., 2006).

New crop varieties: for people or for environments?

In general, plant breeders tend to focus their breeding strategies on regions and agro-ecological conditions: so-called 'target environments'. The idea that people belonging to different social groups (even when working under similar agro-ecological conditions) may have different requirements for seed and varieties, so that we have to target our work not only to natural, but also to social and economic conditions, may be less apparent. In this section, we therefore enter into more detail and describe why we need to explore and integrate both aspects: defining a target environment not only from natural but also from socio-economic perspectives.

General agro-ecological conditions can be described with relatively few parameters, which are usually available from secondary sources, such as general physical maps, soil maps and meteorological data. With this information, we can distinguish agro-ecological zones according to:

- different altitudes;
- different soil types;
- different rainfall patterns;
- availability of irrigation water;
- etc.

Depending on the scale for which this type of information is available, this analysis will result in relatively large zones that appear more or less homogenous. However, this is seldom true in the farmers' reality. Even farmers in relatively favourable agro-ecological regions or irrigated areas often have land that is of poor quality, due to local

constraints such as stones, rocks, gravel or hard subsoil layers, hilly land, or poor quality or limited availability of irrigation water. Therefore, marginal agro-ecological conditions can be found surrounded by more favourable environments, and depending on a farm household's total land area and the location of the fields, these conditions can be of considerable importance (see Box 4.2). The farmers' requirements for seed and varieties depend directly on the conditions present on their land, and on the limitations and constraints they have to face in their daily work. Thus, it is indispensable to complement agro-ecological information from secondary sources with local information, including soil types, irrigation water and typical constraints to agricultural production. Care should be taken to include information from various social groups, as land quality and access to natural resources often vary for different people in a village.

Furthermore, the same natural and agroclimatic conditions can pose different problems and opportunities for people, depending on other resources they possess. For example, soil constraints may have different importance depending on the machinery used by a farmer, and the availability of groundwater for irrigation purposes helps only if a farmer family can afford the irrigation equipment and operation costs. Expensive seed and other costly inputs may not be accessible for poor farmers, so that they have a preference for varieties that can be multiplied on farm and successfully grown under low-input conditions, even in a favourable agro-climatic environment. These examples show how economic factors influence the farmers' needs and preferences regarding crop varieties.

Social factors may be of equal importance. People belonging to different social groups may have different needs, preferences and access to resources. In many cultures, for example, women and men have different responsibilities with regard to farming, nutrition and income generating activities, which may result in different preferences. Ethnic groups, clans or castes may be specialized in certain agricultural activities, such as pastoralism, general farming, horticulture or cultivation of trees, and

BOX 4.2

Soil quality and settlement patterns

In some parts of the world, we can observe some level of coincidence between agro-ecological conditions and settlement patterns, so that distinct social groups live and work under different agro-ecological conditions even in the same village. Examples are:

- Remains of feudal systems: The kings and members of the nobility usually possessed the best lands and the rights to access water and other natural resources. The 'ordinary people' worked on marginal lands.
- Remains of colonization: In the process of colonization, indigenous people were forced to leave their land and settle in less favourable conditions.
- Migration due to wars or disasters: Refugees and other 'newcomers' are often allocated marginal lands that are not used by the original population.

These settlement and land use patterns can persist for generations

Source: Christinck and Weltzien, 2005.

cooperate according to traditional rules and rights.

One practical option to clarify and limit the target environments for a plant breeding programme is to identify with farmers the variety that the new programme needs to replace in order to be successful. In cases where this is possible, the range of distribution of this variety may then be considered the target environment(s) for the new breeding programme. In areas of high varietal diversity, this may not be so evident, and may require more understanding about which varieties or group(s) of varieties play what role in the production system and livelihood of the target group of farmers. In other situations, it may actually be most useful to add a new variety to the spectrum of varieties already grown by farmers, with specific new uses or adaptation characteristics, such as sorghum with good malting qualities to meet the needs of an emerging industry.

In summary, farmers may have different needs and preferences regarding crop varieties and specific traits in relation to their economic situation and their social group(s). Therefore it will be important to develop an understanding of how natural as well as socio-economic factors relate to the farming practices of different farmer groups, particularly in view of their use of varieties and needs for specific traits. The decision about the target group of farmers determines largely which project goals can be achieved, which is decisive for the 'success' of a project. Since the decision on target groups guides many subsequent steps in the priority setting process, it should be a primary concern for plant breeders. Similarly, evidence from impact assessment studies has shown that adoption of new varieties is often limited because the target group and their specific needs and preferences were not adequately considered by breeding programmes (Weltzien *et al*., 2003; Witcombe *et al*., 2005).

4.4.3 Selection criteria

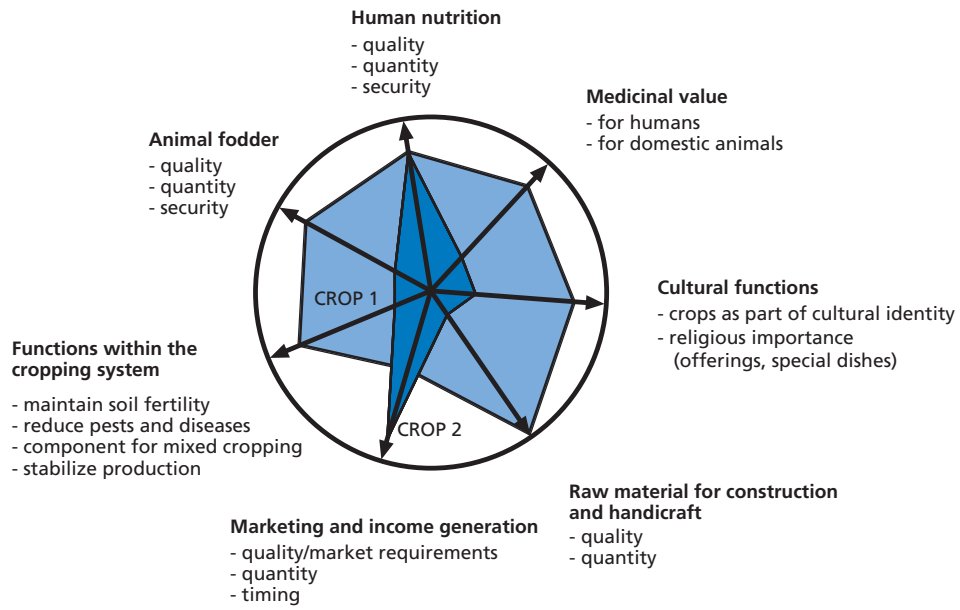
Once the project goals as well as target group and environments are identified, decisions about the type of improvements needed and the selection criteria will come into play. Looking towards future options requires a sound understanding of the situation and the conditions under which newly developed varieties will need to function. This will be the basis for developing new, creative options.

Functions of crop varieties in the farming system and related selection criteria

Crop varieties, particularly those with a long history of cultivation in a given region, are not only adapted to natural conditions, but also to the needs of the people and their cultural preferences. They can fulfil a wide range of functions within the entire system of farming, nutrition and cultural life of a farmer family, and provide important by-products (see Figure 4.2). However, as many rural areas are in a process of rapid socio-economic change, improvements in specific traits can be interesting for the farmers. In most cases, this will depend on the economic importance of this particular trait, and the overall acceptability of the variety with regard to other important traits. This figure can also help us to think about the type of improvements needed to achieve the project goals.

As a first step, we should gain some knowledge on the farmers' variety portfolio, their use of varieties and the strengths and weaknesses of these varieties in relation to functions and project goals. This characterization of varieties should be based on farmers' knowledge and perceptions.

FIGURE 4.2
Functions of crops within a farming system



Crop 1 is a typical multipurpose crop with high importance for most functions (except marketing), whereas crop 2 is a food crop important for nutrition and marketing, but not for other functions mentioned
Source: Christinck, Dhamotharan and Weltzien, 2005.

Various tools for entering into dialogue with farmers on variety characterization and use have been proposed by Christinck, Weltzien and Dhamotharan (2005). Furthermore, understanding farmers' own seed selection and the underlying criteria will give us important keys for the types of improvements farmers are looking for.

Some selection criteria are largely determined by the requirements of adaptation to the target environment, e.g. flowering date, resistances to specific pests and diseases, or to abiotic stresses such as soil acidity. Other selection criteria are determined by the technologies farmers are using, such as ease of harvesting, transportability, manual threshing, or by the requirements of the farming system, e.g. mixed cropping and fodder use. Furthermore, selection criteria may also be related to culinary preferences, such as taste, usefulness for certain preferred dishes, to useful by-products (i.e. construction material) or to market requirements, e.g. grain colour and shape. In most cases these criteria must meet a certain threshold level of acceptability.

Experience from PPB projects has shown that farmers often select for many criteria simultaneously, and in this way can indirectly achieve considerable yield increase. This seems to be mainly related to the farmers' ability to anticipate the performance of certain plant types under specific conditions that are well known to them (Sperling, Loevinsohn and Ntabomvura, 1993; Christinck, vom Brocke and Weltzien, 2000).

However, for professional plant breeders, a detailed evaluation of each and every trait

that might be important for farmers will lead to a dead end. Resources for testing and evaluating new germplasm or breeding material are always limited. The more criteria that are included in a selection programme, the less effort can be spent on each of them, and thus less progress tends to be obtained from selection. Thus, a guiding principle in the choice of selection criteria should be to keep them to the minimum necessary. The more focused and clear the targets for selection, the greater are the chances of achieving them. We find here an excellent option for cooperation between farmers and scientists in a breeding programme. Farmers can more efficiently select those materials that are overall compatible with their situation, farming system and marketing requirements and preferences, whereas scientists can be most effective in assembling appropriate germplasm with the traits desired by the farmer and in selection for a limited number of critical traits.

Heritability of traits and environmental adaptation

Formally trained plant breeders tend to classify traits by the complexity of their genetic control. They differentiate highly heritable traits with simple genetic control from genetically complex traits with low heritability, along a continuum of increasing complexity, and thus decreasing genetic control or heritability (see Chapter 2).

Highly heritable traits with simple genetic control tend to be mostly descriptive traits, such as colours of the grain or other plant parts, hairiness, key aspects of crop duration or flowering date, plant height and some types of disease resistance. While some of these traits are key factors for the adaptation of a variety, such as flowering date or disease resistance, many others are more related to what is intuitively often thought of as a preference: something visual, qualitative and not really associated with productivity or adaptation. Most of these traits could actually be incorporated into existing varieties by backcrossing, if a source for the desired trait, i.e. a gene, exists in the breeders' collection.

Complex traits have a low heritability because their expression is highly influenced by environmental factors, i.e. the conditions in which the variety is grown. Many of these traits also tend to show sizeable amounts of genotype × environment interactions, i.e. the expression of a trait in specific varieties depends on the conditions in which the trait is being evaluated (see Chapter 20). One example would be a variety which responds well to fertilizer; its yield under high fertility conditions could be higher than that of a local variety, whereas the local variety would outperform this variety under low soil fertility conditions. This example shows clearly that identifying yielding ability as a key preferred trait is of little relevance. However, what is important is the specification for which kind of growing conditions a higher yield performance is being sought by farmers. This type of specification is necessary for most of the complex, productivity-related, traits, as their assessment cannot be dissociated from the conditions under which they are evaluated.

Another example of a selection criterion, which is often high on farmers' lists of preferences, but usually very difficult to assess, is drought tolerance. The first problem is that a trait like drought tolerance may mean very different things to farmers, to crop physiologists or to breeders, and would thus entail very different ways of assessing it, from physiological measurements of drought response at the biochemical, plant tissue, plant organ or

whole plant level, through to productivity under specific drought conditions. Practical breeding experience with drought tolerance has shown that it is of key importance to ensure that the crop's water requirements match the periods of water availability in the target production system. It is thus important that the nature of such complex traits of adaptation are well understood before deciding to use them as a focus for selection and variety improvement. Traits that cannot be assessed or evaluated with the necessary precision in the planned project should thus not be included as selection criteria. Before it can become a selection criterion, some research might be necessary to find appropriate ways of assessing or measuring such a trait.

New selection criteria can lead to new options

It could be a 'breakthrough' for farmers if some well known traits of already existing varieties could be improved. However, in some situations, radically new options can emerge if totally new selection criteria are taken into consideration. For example, in regions where crop production has so far been merely subsistence oriented, traits important for food processing industries could lead to new marketing options. Totally different plant types with different growing behaviour, such as extra-short growing cycle or extra-tall plants, could help farmers to diversify their farming systems.

Such extreme changes can often not be envisaged by farmers, if they have no practical experience with such varieties. Thus, it is an important task for plant breeders to find out (together with farmers or based on a thorough understanding of the farming systems) which new options could really be beneficial and interesting for the farmers.

On-farm or on-station evaluations of exotic varieties, excursions to food processing plants and visits to other regions could be a way to start developing radically new options with farmers.

Success from selection

A clear target is essential for the effectiveness of any plant breeding effort. The clearer and the simpler the target, the greater are the chances of achieving it. If the target, and thus the priorities for selection, can be simplified, then the full selection effort can be focused on those key traits. Such targeted selection efforts have a much higher rate of success and of progress from selection than programmes that have to consider multiple and very complex traits as selection criteria. Therefore, investing some time at the beginning into the development of clear priorities for selection can help enormously to increase the overall efficiency of a breeding programme. This is why most PPB programmes put great emphasis on understanding farmers' preferences and needs (Weltzien *et al*., 2003).

Selection priorities may be different for different groups of farmers. Transparency here can help to compare the identified selection priorities once again with the overall project goals, and then decide how (and with which group of farmers) to best achieve them. Tools for discussing different options and trade-offs with farmers will be presented in the last section of this chapter.

4.4.4 Choice of base germplasm

Selection can only be successful if there is sufficient diversity from which to choose. It is thus clear that the selection criteria and the choice of germplasm are intimately linked. Traits for which no genetic variability is available cannot be considered for genetic improvement. Similarly, the extent

of diversity available for selection largely determines the success of the selection programme. This is particularly important in view of the first guiding principle for prioritizing selection criteria, namely to keep criteria to the minimum necessary.

Using local germplasm as breeding parent: a way to increase the acceptability and adaptation of new varieties

One basic approach for keeping the number of selection criteria to a minimum is to identify base germplasm that already has most of the traits expressed at the threshold level or above, but is variable for the major trait targeted for improvement. Many PPB programmes have been very successful in this respect, because they did use the local germplasm and farmers' knowledge of it for this purpose. By using local germplasm, most of the traits for adaptation and use are already expressed at this threshold level, and the novel germplasm can be chosen to introduce new variability specifically for improving one or two key traits, e.g. reducing the period from planting to flowering, or increasing yielding ability, or stover quality, or resistance to a major pest or disease.

Plant breeding and biodiversity conservation

The choice of germplasm is also a key issue for achieving goals related to biodiversity conservation. If used successfully in plant breeding programmes, there are much better chances of 'endangered' germplasm being preserved, compared with other approaches focusing on conservation *per se*. If diversity conservation is a primary goal of a plant breeding programme, a very good understanding of the nature and functions of this diversity for the target group needs to be achieved. Assessing local diversity in a participatory research process can, as such, contribute to raising awareness about the usefulness of this diversity among participating farmers and scientists, and thus increase the chances for future use of this germplasm. However, the goal of increasing biodiversity in farmers' fields does not necessarily require a focus on local and traditional germplasm. Particularly in those regions where a major part of the local diversity is already lost, a plant breeding programme could also be based on material from elsewhere, showing enough diversity in traits that have been identified as useful for the target group of farmers.

Any adoption of new varieties by farmers will change the portfolio of varieties available in a village community. This may provide interesting new options for some farmers, and possibly disadvantages for others. Such developments can often not be anticipated fully. Unintended (negative) outcomes for some farmers can be reduced by ensuring the multiplication and access to seed of the original varieties, for example through strengthening seed exchange networks, institutionalizing seed fairs or community seed banks.

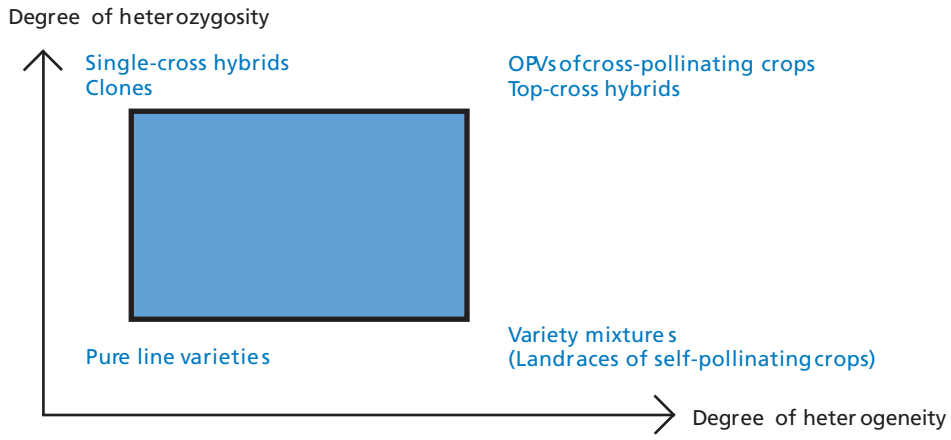
4.4.5 Types of variety

What type of variety will be developed in the course of a plant breeding programme has important implications with regard to the biodiversity in farmers' fields and to the options farmers have to use seed of this variety for re-sowing, selling, exchange and their own breeding activities. These aspects touch the overarching goals of the breeding programme, and are thus important for consideration in the process of priority setting.

Variety types and agrobiodiversity

Varieties can have very different genetic structures; they can differ in the degree

FIGURE 4.3
Degree of heterogeneity and heterozygosity in different types of varieties



Source: Weltzien and Christinck, 2005.

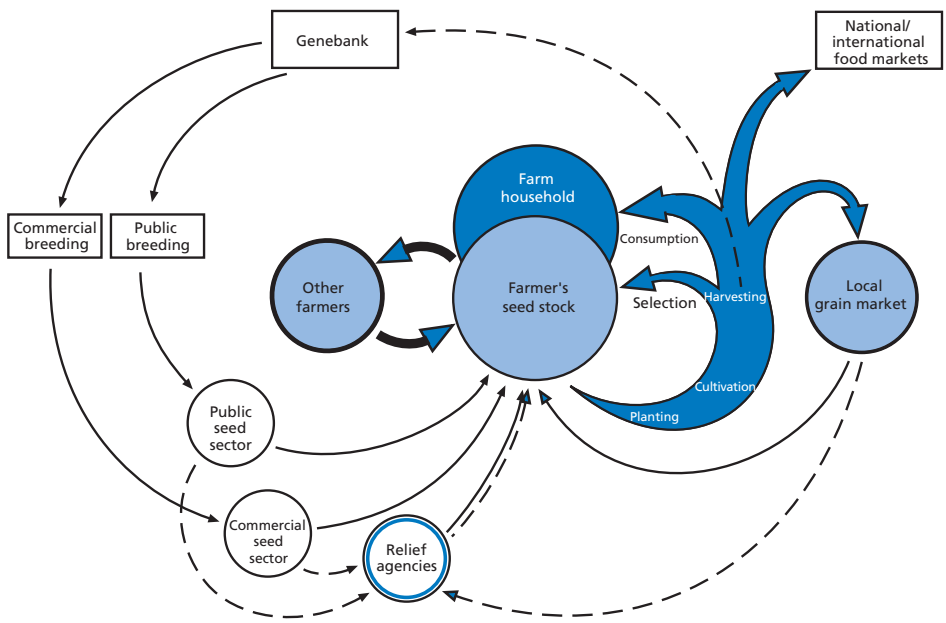
of variability maintained within the variety and in the degree of heterozygosity, with important implications for the ease of reproduction (Figure 4.3).

Pure line varieties of self-pollinated crops are homogenous and homozygous, and could theoretically just be made up of one single genotype that can easily be reproduced. Single-cross hybrids may also be made up of only one genotype (the offspring from a cross between two homozygous parental lines). However, they have very high degrees of heterozygosity and cannot easily be reproduced by farmers. Other types of hybrids will have different levels of diversity within them, such as top-cross hybrids, where one parent is an open-pollinated variety of a cross-pollinated crop. Open-pollinated varieties have a high degree of intravarietal diversity. Heterozygosity is also present in such varieties, depending on the out-crossing rate of the crop and the diversity of alleles for genes in the population. Open-pollinated varieties can be reproduced easily if contamination with pollen from other varieties can be prevented. Variety mixtures (multi-line varieties) or some landraces of self-pollinating crops may be both homozygous and heterogeneous. They are reproducible if natural selection pressures do not differ very much from the conditions under which they were developed, so that specific types or components will not disappear.

Furthermore, a breeding programme could also reach diversity-related goals through developing a number of varieties for specific conditions and uses, and for various user groups. This approach has important implications in the longer term, because it will require a continuous effort to maintain and disseminate all these varieties (see Section 4.5, on Roles and responsibilities of partners).

For the process of priority setting, we have to consider which form and degree of diversity—and of which material—will be required to reach the diversity-related goals of the programme, and how important it is that the seed can be easily reproduced and re-used by the farmers. The latter point is discussed in more detail below.

FIGURE 4.4
Formal and informal dimensions of seed systems and how they may interact; varieties from the formal system may enter the local systems and vice versa



Source: Sperling and Christinck, 2005.

Variety types and farmers' access to seed

The seed channels farmers use for sourcing their seed are normally grouped into two broad seed systems: the formal and the informal seed systems. The latter is also sometimes termed the local, traditional or farmer seed system (see Figure 4.4).

The formal seed system involves a chain of activities that lead to clear products: i.e. certified seed of verified varieties. Thus, the chain usually starts with plant breeding in research institutions or commercial companies, and results in varieties or hybrids intended for formal variety release. Formal regulations aim to maintain varietal identity and purity, as well as to guarantee physical, physiological and sanitary quality. Seed marketing takes place through officially recognized seed outlets, either commercially, or via national agricultural research systems (Louwaars, 1994).

The informal system embraces most of the ways in which farmers themselves produce, disseminate and obtain seed: directly from their own harvest; through barter among friends, neighbours and relatives; and through local grain markets or traders. The same general steps take place in the informal system as in the formal, but they take place as integral parts of farmers' routine grain production rather than as separate activities. Also, rather than be monitored or controlled by government regulations, informal seed sector production is guided by local technical knowledge and standards, and by local social structures and norms, including market forces (McGuire, 2001). Varieties may be landraces or mixed

races, or improved varieties that have made their way into the local system.

Perhaps because of their ability to meet local needs and preferences, informal channels provide most of the seed that small farmers use: it is estimated that somewhere between 80 and 90 percent of total seed sown originates from the informal system, although this varies a lot between different countries and regions, as well as for different crops. A formal seed system does not exist in practice for many local crops or varieties of minor economic importance, whereas it is particularly important in regions where hybrid maize is grown. The relative importance of the formal and informal seed systems also much depends on the seed legislation of the respective country. Very restrictive seed laws have practically abolished the informal seed system in some countries, whereas in others the legislative framework allows for the co-existence of both systems.

Professional plant breeders are usually members of formal institutions (public or private), so that formal channels of seed production and dissemination are the 'normal' route through which newly developed varieties find their way to farmers' fields. However, the formal and the informal systems have both comparative advantages and disadvantages for variety diffusion, and often address different client groups. Considering these differences could form part of an active strategy for effective variety diffusion in relation to the goals of the breeding programme. For example, the informal seed system has various advantages for poor farmers, as the seed price is usually lower and the modes of payment flexible. If poor farmers' access to new varieties is a goal of the breeding programme, variety diffusion through the informal system could be a good option for reaching this goal. At the same time, the informal system often builds on traditional rules and forms of cooperation in village communities, including cooperation among different wealth and ethnic groups. Thus, detailed knowledge of the seed systems and how they are related to different groups of farmers is required for developing such strategies (Sperling and Christinck, 2005).

The type of variety that will be developed, and how it can be reproduced and maintained by farmers, is thus a very important consideration for a breeding programme, particularly in situations where the formal system alone cannot serve the target groups of farmers.

4.5 ROLES AND RESPONSIBILITIES OF PARTNERS

4.5.1 Cooperation between different organizations and stakeholders

Plant breeding is increasingly being done as a partnership among different stakeholders: individuals, groups, organizations who share an interest in using and improving crops. It is thus clear that the discussion about roles and responsibilities of the different partners is at the heart of such plant breeding projects, and is thus a critical issue in the priority-setting process.

The 'history' of a project (who took the initiative and for what interest?) appears to play an important role in this regard. It makes a difference whether one organization initiated the project and organized the major part of the resources, and then sought potential partners, or whether it was a joint initiative from the outset. The present structure of international agricultural research, particularly with regard to funding and accountability, potentially poses problems for cooperative research that involves very different types of institutions. This is due to the large differences between organizations regarding

their access to external funding, and the fact that the institution that successfully acquires funds is usually alone accountable towards the donors, which often impedes a real sharing of project responsibilities among the partners (Kolanoski, 2003).

Notwithstanding, for the process of priority setting, it appears recommendable to look deeper into the key skills and resources (material and non-material) each partner or partner organization has to offer for reaching the identified project goals, for example, with regard to several issues:

- Overall project management, including decision-making processes, monitoring and evaluation, reporting, public relations work at different levels, fund acquisition and management.
- Planning and implementation of practical project activities, such as trial management and data analysis, or seed production and dissemination.
- Training and skill-building activities.

On this basis, contracts between the various institutions could be negotiated, which include tasks and duties with regard to the project, as well as the distribution of funds and resources among the partners. Furthermore, a pre-agreed procedure for mediation or a conciliation board should be foreseen in view of future cases of disagreement that might crop up between the partners.

4.5.2 Cooperation between farmers and scientists

In projects initiated by formal-sector breeding programmes, which are mostly concerned with the traditional goals of breeding programmes, such as productivity increases and possibly changes in policies for variety release or seed diffusion, most of the decision-making about the project tends to be initially in the hands of the scientists. The farmers often play a rather more consultative role, giving input into variety evaluation, prioritization of selection criteria, and the necessary insights required for focusing the project. However, as partners gain experience, and the scale at which the project operates increases, projects tend to develop towards a strengthened role for farmers or their organizations, especially in terms of selection decisions and variety evaluation.

If farmers, especially a farmer organization, initiate a plant breeding project, it tends to be clear that they seek specific support or input from scientists to find solutions to problems already well identified. In addition to specific technical support, scientists can make contributions to building farmers' skills with respect to obtaining new germplasm; crop biology or physiology; specific plant breeding activities, such as crossing; variety evaluation; and interpretation of results. In such situations, it is clear that the role of scientists is primarily a consultative one, while key decisions are taken by the farmers or their organizations.

In situations where farmers are not well organized, but project partners have identified farmer empowerment and skill building as a project goal, the project may invest major resources in the establishment of farmer organizations, committees or groups, which can then manage more of the key breeding activities, and over time become the primary decision-makers, as their skills and organizations grow. In such a scenario, the role of the researchers may change considerably over time, especially in terms of the management of trials, such as decisions about which materials to continue with or to abandon, or which priorities for selection to add to the project. Usually these changes are also accompanied by a change in the scale of the project. There

is thus an increase in not only the skills of the farmers, but also in the number of farmers and of villages, and thus possibly the number of crops, target environments and priorities for selection.

In any case, a reflection on the different approaches and skills of farmers and researchers could be a valuable basis for priority setting with regard to roles and responsibilities of partners in a breeding project. Farmer experimentation is in various respects different from the experimental designs usually applied by scientists, and has been described by a number of authors (Johnson, 1972; ILEIA, 2000; Leeuwis, 2004; Reijntjes and Waters-Bayer, 2001; Saad, 2002). Respecting and learning from farmer's informal experimentation and evaluation approaches could lead to valuable insights and innovations, and could thus be assigned a role of its own in a participatory breeding project.

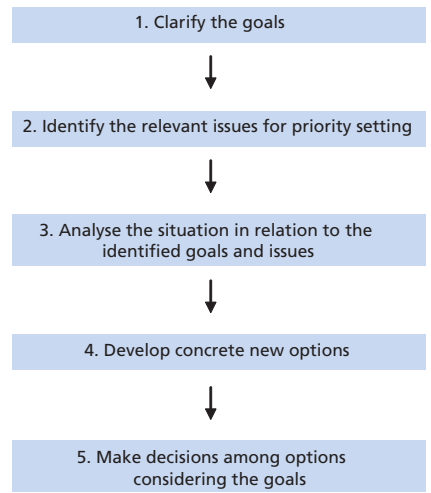
4.5.3 Decentralized breeding programmes

Breeding programmes that aim at exploiting local adaptation or increasing diversity in farmers' fields usually have to be organized in a strongly decentralized manner, as a number of varieties will have to be tested, multiplied and distributed among a limited number of users. In such cases, the responsibilities should also be shared from the outset to ensure the sustainability of such activities. Skill building, training and institutional development may be important elements in such projects, and could support farmers to manage locally preferred varieties by themselves.

4.6 PRACTICAL METHODS FOR PRIORITY SETTING

Priority setting for plant breeding programmes is, as such, not much different from other situations, and includes a number of steps (Figure 4.5).

FIGURE 4.5
Steps for priority setting in a plant breeding programme



Before examining some practical tools, we will briefly refer to each of the aforementioned steps.

4.6.1 Clarifying goals

As indicated earlier in this chapter, plant breeding programmes can have a variety of goals, of very different natures. It is important that all the options are discussed with the partners, and that a common vision is achieved for each project and for the programme as a whole. It is important that discussions about the goals are held regularly to ensure that the goals remain relevant, and that they remain clear, evident and important to all partners involved in the programme.

4.6.2 Identify the relevant issues for priority setting

The critical issues for priority setting in a plant breeding programme have been

outlined in the first part of this chapter (see Figure 4.1). All these issues need to be addressed by any plant breeding programme, but there may not always be viable alternatives to chose from. Besides, the goals of a breeding programme can change over time, reflecting the particular context or situation; thus, priorities need to be reviewed regularly.

For the purpose of identifying relevant options for the key issues, it may be helpful to examine the chances of success with regard to each of the goals. This could be done during a planning workshop, or also in the form of an e-mail discussion for those partners who are using this communication technology. Furthermore, it is likely that new options and insights emerge in the course of the practical project activities. Therefore, the process of priority setting should be implemented in such a way that insights and challenges can be addressed at regular intervals, and then be integrated into previous concepts.

4.6.3 Situation analysis

Realistic new options or technologies require a good knowledge of the situation under which they are intended to function, including the needs and preferences of the potential users. Client-orientation is a key concept in the general economy, and increasingly also in plant breeding (Witcombe *et al*., 2005). In the past, client-orientation was sometimes under-developed in plant breeding, particularly as far as resource-poor farmers in marginal areas were concerned. A basic understanding of the complexity of farming systems in such situations, as well as their dependency on environmental adaptation and biodiversity, has now been developing, mainly since the mid-1990s.

The situation analysis for a plant breeding programme should focus on those issues required to effectively reach the goals of the breeding programme. In general, it will have to include the following issues:

- agro-ecological conditions;
- socio-economic conditions, including marketing of crop-based products;
- the farming system, actual processes of change and main limitations;
- farmers' use of varieties and their seed management;
- seed system analysis; and
- specific varietal needs and preferences of the target group(s).

The situation analysis could include the following steps:

1. Review secondary sources.
2. Consult local experts, key people with good knowledge of the potential target area(s).
3. Visit potential target areas and consult farmers belonging to different social and wealth groups.
4. Structure and compile the information for further planning.

Experience gained in a number of PPB projects has shown that participatory communication tools, such as semi-structured or informal interviews, focus-group discussions, wealth ranking, transect walks, time lines, mapping, classification and ranking exercises, can be extremely useful for providing a good basis for further planning. The particular strength of such communication tools is that they facilitate direct dialogue between farmers and researchers, and can help to develop a common understanding of the situation, as well as of the main constraints and needs. Practical guidelines for conducting such a situation analysis, particularly for plant breeding projects, have been suggested by Christinck, Weltzien and Hoffmann (2005). Furthermore, much inspiration can be gained from general guides and

BOX 4.3

Web sites on participatory research methods

Sources of information and training materials are listed below. We concentrate here on those publications that are available via the Internet, often for free download.

1. The Web sites of FAO (www.fao.org) and the World Bank (www.worldbank.org) contain sections on publications for download and/or purchase (search for "participation" or "PRA").
2. Further publications may be found via the online bookshop of UNEP (United Nations Environment Programme) www.earthprint.com in the section on Participation and training.
3. An introductory guide to participatory learning approaches can be downloaded free of cost from the GTZ homepage: Schönhuth, M. & Kievelitz, U. 1994. Participatory Learning Approaches. Rapid rural appraisal, Participatory Appraisal – an introductory guide. http://www2.gtz.de/dokumente/bib/95-0930.pdf Other language versions (Spanish, French) are available upon request. More specific publications on participatory research and learning are accessible for download from: http://www.gtz.de/de/themen/uebergreifende-themen/partizipation/15201.htm (Accessed 12 September 2008).
4. Participatory Learning and Action (formerly PLA Notes) is a series on Participatory Learning and Action (Methods and Approaches), accessible through the IIED homepage (International Institute for Environment and Development, London, UK): http://www.iied.org/NR/agbioliv/pla_notes/about.html#a (Accessed 12 September 2008).
5. The Programme for Participatory Research and Gender Analysis (PRGA) has a Web site with a series of publications and resources, including a listing of cases for participatory plant breeding. (www.prgaprogramme.org)
6. Reading University, UK, maintains a Web site with training materials and resources focusing on the statistical analysis of data from participatory research activities: http://www.reading.ac.uk/ssc/workareas/participation.html (Accessed 12 September 2008).

publications on participatory research (see Box 4.3).

The use of qualitative social science methods for conducting studies in plant breeding projects has long been debated. Plant breeders are used to working with large numbers of accessions and observations on various trial sites, so that statistical data analysis is a standard method in this field of research. However, results from informal qualitative research are not necessarily less precise (only there are no numerical estimates of how precise). For many purposes in a plant breeding programme, and particularly in the initial phase, the main focus would be to initiate dialogue and identify potential partners. Often, it is possible to start with rather informal and qualitative research methods, in order to identify the main issues of relevance, and to use this knowledge later for more formal studies, if required. There are also increasing efforts to combine qualitative with quantitative, and informal with more

formal methods (Bellon and Reeves, 2002; Abbeyasekera, 2002).

4.6.4 Develop concrete new options

Developing new options for varieties requires creativity and good knowledge of the conditions under which a new variety will have to 'function'. It also requires good knowledge of the available diversity of the crop. Similarly, it may require detailed understanding of options for new crop uses, and for marketing of crop products, possibly new ones. Traditionally, plant breeders have done this based on their own understanding of the farmers' reality, especially as many of the early private plant breeders were farmers themselves. Nowadays, when plant breeders work on a national, regional or international scale, the development of new options for variety development, and seed distribution requires working creatively with farmers and other project partners from various institutions and disciplines. This is usually a continuing process, and thus the project or programme should be organized in such a way that regular reviews of alternative new options can take place.

4.6.5 Making decisions among various options considering the goals

Making choices between the different options needs to be forward looking, based on the identified project goals, and on chances for success. Different stakeholders and partners will have different perspectives, and thus their choices and preferences for specific options will vary. Hence it is important that the process of making decisions among an array of options is transparent, and that the roles and responsibilities of the different partners in the decision-making process are agreed. Ranking exercises are ideal tools for taking decisions based on transparent criteria. Participants may make their decision first, and then explain the reasons for their choice. Implicit reasons can thus be made explicit and transparent. More refined tools, which can consider several criteria simultaneously, may be used once the key criteria are agreed.

4.6.6 Tools for farmer participation in the priority-setting process

In this last section, we present a series of tools that have been used successfully in one or more of the steps of the priority-setting process outlined above. Some may be used only for one specific step in the decision-making process; others may apply to several of the steps. Many of the tools have been successfully used with farmers for the identification of critical selection criteria. The tools we choose to describe are primarily those that can be used with a wide variety of partners, specifically with farmers, but also with those who may have very little time, may not be literate, but may have a profound knowledge of their culture and crop related issues. Many of the tools are described in more detail and with more examples in other sources, sometimes in other contexts. Some good source materials are cited and listed. In most instances, one would apply not only a single tool, but several; it is advisable to vary the tools for different steps of the priority-setting process, and also for the purpose of verifying and increasing the reliability of previous results and hypotheses.

Facilitated discussions on goals, issues and criteria

Invite all relevant project partners to a meeting on discussing goals for a new plant breeding programme. As the outcomes will possibly depend on the circle of persons invited, the invitation list should be carefully thought out. Furthermore, particularly if

farmers are involved, the language, the general 'setting' and the working style (are all participants literate?) should be considered with awareness.

Depending on the number of participants, there are various options for facilitating such a meeting. One option would be that the participants from each organization are asked to prepare a short presentation, which would include a sort of problem analysis based on their own experience and viewpoints, and should propose goals and priorities. After the presentation, the main goals mentioned in the presentation would be documented on a board. In this manner, there would be a preliminary list of goals at the end, which could then be further discussed.

Another way would be to start with a 'brain-storming session' or open discussion on goals, and to document the proposed goals on a board for further discussion.

There should then be time to discuss these goals in more detail and clarify what they imply. Very often, it helps if the participants are asked what kind of indicators they would suggest as a 'measurement' of whether the future project activities would be successful or not in reaching these goals. Such indicators could thus also be useful for future monitoring and evaluation meetings.

It is of particular importance to identify potentially conflicting goals, or utopian goals. In such cases, the group could try to weigh up different goals, or to make utopian goals more realistic and situation-specific. In general, it is of course much easier to reach a few clear goals with high priority on the agenda of all participants, than a long list of potentially conflicting goals. At the same time, the discussion of goals can anticipate many problems that might occur in the course of a plant breeding programme, particularly if many partners are involved.

The meeting could then finish by prioritizing the suggested goals, such as through a simple ranking or scoring exercise (see below).

In any case, such discussions on goals should be regarded as preliminary results. Many goals are not easily expressed and are closely related to individual or culture-specific values. Moreover, goals may evolve in the course of the project activities. It is thus recommended that this discussion be repeated later, for example after completing the situation analysis (see Section 4.6.3, above), and particularly in view of the question of whether the goals are really relevant for the target group. Regular discussions on goals and indicators, for example at the beginning of each new working phase, or in a general planning meeting, can be rewarding if a good facilitator helps to ensure productive outcomes.

SWOT analysis

A discussion about the overall goals and more specific priorities involving key actors or stakeholders can be structured in the format of an analysis of the present situation of the crop under discussion and the development of future varietal options. A strategic planning tool for this type of analysis is SWOT analysis, a structured discussion on **S**trengths, **W**eaknesses, **O**pportunities and **T**hreats. This discussion could be held as part of a project planning workshop, for example on the topic: 'Farmers' groundnut varieties for the dry areas of Senegal', or any other crop and region.

The participants, either individually or in small groups, are first asked to think about the strengths of the situation under discussion. The results should be documented on a board or piece of paper

(for later presentation to the whole group). In the following steps, the participants also discuss weaknesses, opportunities and threats. The results should be documented visually on a board, and could then serve as a starting point for discussion on goals and priorities of a breeding programme (see also Weltzien, 2005).

Recurrent feedback discussions

Successful project work depends on good interaction between partners, e.g. researchers from various institutions, farmers, and extension or NGO personnel. Feedback discussions during which the different partners openly exchange their views and experiences with specific project activities should be held at regular intervals. These discussions about what worked well, or which problems or opportunities arose, are the basis for reviewing the project priorities in an evolving partnership between very different types of organization. While there may not necessarily be a fixed framework for such discussions, they are instrumental in refining project priorities and in the evolution of the overall goals of a project and a partnership. Participatory Monitoring and Evaluation (PM&E) would be a more 'institutionalized' way of conducting such feedback discussions (Germann, Gohl and Schwarz, 1996).

Simple scoring exercises

If you wish to set priorities among a number of possible goals, criteria, problems or issues in a formal way, simple scoring exercises can be applied. This requires that a tentative list of goals and criteria is already established.

These goals should be written on a board or be represented visually in some form (graphically or as text). All participants get a predefined number of counters, such as pebbles, paper pieces, adhesive dots, etc., and are asked to put their counter next to those goals with the highest priority for them. The goals should be well understood for this exercise, and the rules explained carefully. Generally, each participant should have fewer counters than goals, so that a real decision has to be taken. It should be clarified whether it is allowed to assemble all counters at one goal, the one perceived to be more important than any other, or if only one counter can be placed for each goal. In this manner, you will obtain a clear result within a relatively short time—a result on which further discussions can be based.

Ideal variety

Invite a small group of participants, preferably 2 to 4, with whom you have already discussed variety trials or the importance of specific traits in particular. Larger groups could split up into separate working groups and later present their results to the whole group. Invite each participant to think about what a really good variety of the crop on which you are working could look like, referring to the previous discussions you have had. Focus group discussion, where different groups represent farmers with differing backgrounds, farming situations, gender, ethnic groups, etc., can reveal underlying differing needs.

Ask the participants to think about all the characters that a good variety of millet, cowpea, etc., should have, to be useful for them. The traits mentioned by the participants should be written on cards, or the participants should find symbols for visual representation; the cards should then be placed vertically in a column. Make sure that everybody contributes and that all the important traits are mentioned. In the course of the exercise, you may also suggest some trait(s) if you are particularly

interested in sparking off a discussion on the relative importance of some new traits. Once all the traits have been identified, you can then ask the farmers to discuss the importance of the trait for a new variety that would be better than the existing ones. To indicate the level of importance of each trait the farmers could distribute a fixed total number of tokens between the traits they (and you) have mentioned. The more important a trait, the more tokens it receives. Traits that are not required should get no tokens, and can be eliminated.

It is best to facilitate this discussion in such a way that the participants primarily discuss among themselves about each trait; for example, how early the ideal variety should be, or how much grain yield in relation to stover yield they think would be useful. The difficulty is to try to keep the discussion within the realm of biological reality, i.e. **not** only grain yield, increased 10-fold with half the growth duration of existing varieties.

Create scenarios

Scenarios can be used to find out whether certain concrete new options are attractive for the target group(s) of farmers. This approach is particularly useful in the case of complex or interrelated trait combinations. For this purpose, we need seed and plant material in which these new trait combinations are already expressed (i.e. exotic or experimental varieties).

By simulating a situation in which farmers have to take a decision between various complex options, immediately followed by an interview about the reasons, then important criteria and trade-offs may be revealed. Furthermore, this is also a way to study whether and why people belonging to different groups take different decisions regarding the proposed options.

Scenarios are only useful if the farmers' reality is reasonably well understood. If the options or choices presented to farmers are not realistic, the responses cannot be expected to be realistic either.

Example 1: Seed shop exercise

The scenario is that the farmer who has no seed of this crop at the time of sowing enters into a seed shop and has to choose among a set of varieties with different properties.

For this purpose, seed of different varieties, local and introduced, is displayed in the 'shop', so that the farmers can see and touch the seed. Variety names, plant samples or drawings of the plant type can provide additional information. If you really plan to give the seed to the farmers after the exercise, small packages in sufficient number should be prepared.

The farmers are asked to enter the 'shop' one by one, take their decision and leave the 'shop'; an interview on the reasons for their choice will be conducted immediately after leaving the 'shop'.

The rules of the exercise should be made very clear at the beginning, particularly concerning questions such as whether the farmers will really get seed of the preferred variety, how much, at what time (in the 'shop' or afterwards) and from whom. Such rules potentially influence the result. They should be carefully considered beforehand and then announced very clearly to the participating farmers.

Example 2: Simulating plant selection in a 'field'

The scenario here is that a farmer selects plants from a 'field'. This is very close to the farmers' reality in most cases. A further advantage of this scenario is that many different traits, which may be relevant for the adaptation to specific conditions, different

uses or situations, will be included into the farmer's decision-making.

A small plot or grow-out of a variety mixture or broad-based population will be required, which shows variability with regard to all traits in which the farmers or the plant breeders are interested (known from previous exercises).

The participating farmers are invited individually or in groups to the 'field'. They are then asked to mark with a coloured ribbon or tag a certain number of plants that they would select for growing in their field. Alternatively, the farmers could be asked to cut the plants from the plot for further evaluation. Interviews on the choices taken by the participants could follow.

Simple ranking

If decisions have to be taken among few options (2 to 5), write the options on paper cards or represent them visually with photographs, drawings or real objects. The options and what they imply should be very clear to the participants. Ask a person or small group to put the cards or objects in an order of preference, starting with the best, the second best, third best, etc. Then ask for reasons and criteria used. A detailed description and training exercises can be found in Guerrero, Ashby and Gracia (1993).

Pair-wise ranking

This exercise works well with up to six items or options. The participants are asked to make pair comparisons, indicating which alternative is better, and why. This exercise often results in an exact description of the conditions under which the alternatives work well or otherwise. This exercise has proven very useful for discussions about selection criteria and farmers' preferences, and is explained in more detail by Weltzien and Christinck (2005).

Matrix ranking

Matrix ranking can provide more detailed insights into the advantages or disadvantages of various options. The ranking criteria have to be defined beforehand. Pair-wise ranking or the Ideal Variety exercises could be used to identify criteria for further discussion and variety evaluation. In a planning workshop, the different options or scenarios to be ranked can be related directly to the project goals, or to criteria that are related to the project goals; for example, if income generation through processing is one of the project goals, some of the ranking criteria could be concrete advantages for processing and marketing.

The matrix could be prepared on a large sheet of paper or on the ground. The visual or text representations of the different options to be ranked are usually placed vertically in a row, with the criteria or aspects in a horizontal row. The participants are then asked to rank all options for the first criterion by placing counters (adhesive markers if done on paper, otherwise pebbles, large seeds, etc.). There should be clear rules for placing counters (i.e. only one counter for the option that fulfils best this criterion; or a certain number of counters for the best, second best, etc.).

If you assign a number to each participant, and write the number on the counters used by this person, the result could be useful for further analysis (who preferred which option, and why). Thus matrix ranking needs some efforts for preparation, but can then deliver very detailed results, especially for identifying selection criteria, user groups and target growing conditions.

Scoring exercises

Scores are frequently used by breeders to assess newly-created varieties and breeding

lines. A similar approach can also be pursued with farmers.

Scores indicate a certain level of performance or expression of a trait. For example, the early vigour of varieties could be assessed using a score, where 5 indicates that a variety is extremely vigorous, 4 = very vigorous, 3 = vigorous, 2 = less vigorous and 1 = not vigorous, or weak. Thus scoring applies a fixed scale, as a tool for assessing potentially a large number of new varieties or other options.

There is a fundamental difference between scores and ranks, which can have far-reaching implications. For example, ranking puts varieties in the order of performance or expression of a specific trait. The best variety could actually have a fairly poor performance, if all the other varieties are still worse. The differences between varieties could be very small, but they may lead to different ranks. Ranks do not have an underlying scale, and thus quantitative analysis is more difficult. Ranking can only be done meaningfully with a small set of varieties (not more than seven) (Coe, 2002; Weltzien and Christinck, 2005).

Discussions on the reasons for giving a particular score to a variety will reveal the underlying criteria. It is furthermore possible to compare the scores given by different groups of farmers (gender groups, people from different villages, etc.).

Practically, scoring exercises can be realized in the field in various ways. Literate participants can enter scores (= numbers) in a previously prepared evaluation form. Alternatively, one can use counters (stones, pebbles, paper pieces), which have to be put into a basket, box or bag near the scored plot. More detailed descriptions and examples can be found in Weltzien and Christinck (2005).

Discussions with farmers about their scoring will lead to a better understanding of selection criteria, preferences of specific user groups or for target growing conditions, market demands, etc.

Other tools used for priority setting

The tools described above are explained in more detail in various training manuals and handbooks (Box 4.4) for farmer participatory rural appraisals. Economists tend to use

BOX 4.4

Training materials and books on participatory research methodologies in plant breeding projects

1. Bellon, M.R. & Reeves, J. 2002. *Quantitative analysis of data from participatory methods in plant breeding.* Mexico, CIMMYT.
2. Christinck, A., Weltzien E. & Hoffmann, V. 2005. *Setting breeding objectives and developing seed systems with farmers. A handbook for practical use in participatory plant breeding projects.* Margraf Publishers, Weikersheim, Germany, and CTA, Wageningen, Netherlands.
3. IPRA & CIAT. 1991. *Farmer evaluations of technology: Methodology for open-ended evaluation.* Instructional Unit No. 1. IPRA, CIAT, Cali, Colombia.
4. Guerrero, M.P., Ashby, J.A. & Gracia, T. 1993. *Farmer evaluations of technology: Preference ranking.* Instructional Unit No. 2. IPRA, CIAT, Cali, Colombia.
5. Cleveland, D.A. & Soleri, D. 2002. *Farmers, scientists and plant breeding: Integrating knowledge and practice.* Wallingford, UK, CABI.

other tools, such as Decision Trees, Grid Analysis or Hedonic Pricing Models, for priority setting and the identification of specific selection criteria. These tools have rarely been applied specifically to plant breeding programmes, with the important exception of the hedonic pricing model, which has been used in a number of instances (e.g. Dalton, 2004; Faye *et al*., 2004). These quantitative analytical tools can also be used to analyse data from specifically set up scenarios, or from ranking or scoring exercises. More examples for combining qualitative and quantitative tools can be found in Bellon and Reeves (2002) or Barahona and Levy (2002).

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