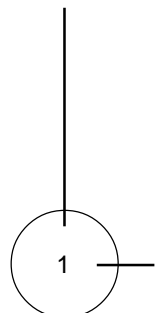# Breeding for Drought and Nitrogen Stress Tolerance in Maize

M. Bänzinger, G.O. Edmeades, D. Beck, and M. Bellon

Breeding for Drought and Nitrogen Stress Tolerance in Maize

From Theory to Practice

M. Bänziger, G.O. Edmeades, D. Beck, and M. Bellon

1

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Abstract: Targeted initially for maize breeders in sub-Saharan Africa, the content is based on a successful approach developed at CIMMYT for improving the tolerance of maize to drought and low nitrogen stress. Intended as a supplement to a course for breeders and agronomists of at least BSc level, it deals with the effects of water and N deficits on the maize plant, the level of yield increases to be expected from selection, factors that affect the severity of drought and low N stress in maize, selecting suitable fields for drought and low N screening, managing uniform stress in drought or low N experiments, designing effective experiments and field layouts for stress trials, the choice and analysis of data, and the use of drought and low N screening in a normal breeding program.
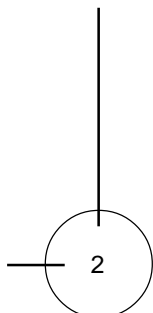
Preface

This manual was developed initially for maize breeders in sub-Saharan Africa, as part of a regional effort to improve the tolerance of maize in southern and eastern Africa to drought and low N stress. The content is based on a successful approach developed at CIMMYT for improving stress tolerance in tropical maize—an approach born of research begun at the center in the early 1980s. Credit belongs to all contributing researchers. Suggestions for corrections and additions should be directed to the authors.

The manual is intended to supplement a course targeted to breeders and agronomists of at least BSc level and which enables them to:

- Understand the effects of water and N deficits on the maize plant.
- Understand the level of yield increases to be expected from selection.
- Understand factors that affect the severity of drought and low N stress in maize.
- Select fields suitable for drought and low N screening.
- Manage uniform stress in drought or low N experiments.
- Design suitable experiments and field layouts for stress trials.
- Become aware of the data that should be taken in drought and low N screening trials.
- Analyze data from stress trials.
- Use drought and low N screening effectively in a maize breeding program.

Contents

1 Introduction:
Why breed for drought and low N tolerance?

1.1 Conceptual framework - Breeding

To understand the advantages of a targeted drought and low N breeding program, recall that simple elements determine progress in breeding programs.

A breeder creates new gene combinations and useful variability among genotypes by **intercrossing** parents that possess desirable characteristics or by **introducing new germplasm from other breedings programs**. This variability is then narrowed by **selection** of the few genotypes that perform best in the target environment.

According to Falconer (1989), a breeder makes the most **selection progress** when

- Differences (*i.e.*, **genetic variance**) among genotypes are large.
- **Selection intensity** is high; i.e., only a small proportion of genotypes is selected.
- **Heritability** is high; that is, traits that are valuable in the target environment can be assessed precisely in the genotypes evaluated and are transmitted to the offspring of these genotypes.

Breeders usually use a **step-wise selection procedure** to identify the best performing genotypes, given limited resources. First, many genotypes are evaluated with few replicates (perhaps even no replicates at all) and at few sites (**screening**). Next, the more successful genotypes or their descendants are evaluated with more replicates and at more sites (**testing**). With each selection decision, the breeder reduces both the number of genotypes and the variation among genotypes, mainly by eliminating the poor-performing fraction.

1.2 Conventional approaches to improving the drought and low N tolerance of maize

Most maize breeders use the screening phase to select for yield potential, resistance to diseases and insect pests, and desirable grain and plant type. Only at the advanced testing stage, when relatively few genotypes remain, are entries evaluated as well under abiotic stress. At this stage, selection intensity is customarily low and progress in breeding for tolerance to abiotic stress is therefore poor.

There are several reasons for plant breeders' apprehensions about selecting under abiotic stress at earlier breeding stages**:**

- Heritabilities and genetic variances for grain yield usually decrease under abiotic stress as yield levels fall. Differences between entries are often non-significant, and expected selection gains are less than under conditions where yields are high.
- Because of the high genotype x environment interactions involved, stressed experiments often produce rankings that differ significantly from one experiment to another, making it difficult to identify the best germplasm.

- Breeders expect that selection under high-yielding conditions will also increase grain yields under abiotic stress conditions.

- In developing countries, farmers in high-yielding, high-input conditions are usually more attractive targets for the private seed sector than the 'average', (often) resource-poor farmer, and commercial sector breeders often ignore abiotic stress-tolerance for this reason. Public sector breeders are influenced by this viewpoint, even though their responsibilities and target environments usually include areas not served by the private sector.

1.3 Conventional approaches challenged

Based on extensive research at CIMMYT, it appears that many of these apprehensions can be overcome, and it is actually possible to make relatively rapid progress in breeding for improved yield under favorable and stress conditions by including extensive screening under abiotic stress conditions. The following arguments underpin this concept:

- If the target environment is commonly affected by abiotic stresses, then the fact that selection gains in an unstressed target environment are higher than under stress is of little or no help in improving yield in the target environment. **If abiotic stresses are the major feature of the target area, the breeder should aim at improving yields for these conditions.** Generally, maize breeding methodologies in the tropics have been influenced strongly by experience from maize breeding in temperate areas. Maize in temperate environments is generally grown under relatively stress-free conditions and on-farm yields approach those obtained on experiment stations, averaging around 7.5 t/ha in a country such as the USA. On the contrary, in tropical environments maize is frequently stressed and on-farm yields fall far below those obtained on breeding stations. Thus, in the tropics selection under high-yielding conditions may not be the best way to increase yields in farmers' fields.

- No breeder would expect to improve disease resistance in maize by selecting in a virtually disease-free environment, yet breeders routinely expect to increase the tolerance of their varieties to drought and low N stress by selecting mainly in high potential environments. This strategy might work, if yields under stressed and favorable conditions were determined by the same plant characteristics. However, as stress levels rise from lack of water or N, different plant characteristics affect yield and genotype-by-stress interactions become significant (Chapter 2).

- When genotypes are is selected under favorable conditions, much useful genetic variation for stress tolerance may be lost. This variation cannot be replaced simply by multilocation testing that exposes a few varieties to stressed conditions at latter stages in breeding. In contrast, a method for reliably detecting abiotic stress tolerance in a large collection of maize genotypes will increase chances of making significant progress in breeding for this trait.

- Over the past 20 years, researchers at CIMMYT have improved maize germplasm for drought and low N tolerance using an approach that is probably unique. Large populations were screened under carefully managed drought or low N stresses so that genetic variation for tolerance was revealed to the greatest extent possible. The

selection gains realized have been considerable — 100 kg/ha/yr under stress conditions — and are well documented (Bänziger et al. 1998; Bolaños and Edmeades 1993a; 1993b; 1996; Bolaños et al. 1993; Byrne et al. 1995; Chapman and Edmeades 1999; Edmeades et al. 1997a; 1997b; 1997c; 1997d; 1997e; 1999; Lafitte and Edmeades 1994a; 1994b; 1994c).

- Maize crops in the tropics are continually exposed to drought and N stress. The incidence of stress may increase, due partly to global climate changes, partly to the displacement of maize to more difficult production environments by high value crops, and partly to declines in soil organic matter, reducing soil fertility and water holding capacity. At the micro level, fertility and water availability vary greatly within many farmers' fields. **This means that a single variety must be able to withstand a wide range of drought stress and nitrogen availability** – a need that is more pronounced in the tropics than in higher input fields of temperate areas.

1.4 The challenge of breeding for drought and low N tolerance

The challenge of breeding for drought and low N tolerance is to find ways of guaranteeing good selection progress. Going back to the conceptual framework, a breeder needs to

- Have useful variation genotypes germplasm in characteristics that confer drought and low N tolerance.

- Be able to assess precisely drought and low N tolerance under conditions that are relevant to the target environment.

- Be able to apply a high selection intensity when selecting for drought and low N tolerance.

Achieving this requires an understanding of the maize crop's behavior under drought and low N stress, the use of stress management, a suite of useful secondary traits that relate to yield under stress, improved statistical designs for use during selection, and an appropriate choice of germplasm and breeding schemes.

2 Maize under drought and low N stress

2.1 Conceptual framework - Physiology

2.1.1 Grain yield as determined by radiation

Plants are complicated systems, and there are many factors that affect yield. One major determinant of yield is radiation capture. Under conditions of plentiful water and N, grain yield (GY) can be considered as:

GY　=　RAD x %RI x GLD x RUE] x HI　　　　　[1]

where RAD　=　incident radiation per day (e.g, 20 MJ/m^2, or 2 x 10^5 MJ/ha)

%RI　=　fraction of incident radiation intercepted by green leaves (e.g., 45% over the crop life cycle)

GLD　=　green leaf duration, or number of days leaves remain green (e.g., 100 days)

RUE　=　radiation use efficiency (e.g., 2 g per MJ, or 2 x 10^{-6} t/MJ)

HI　=　harvest index (proportion of shoot dry matter that is grain; e.g., 0.40)

The term in brackets in Equation 1 represents the total shoot dry matter production and HI is the partitioning coefficient to grain. In our example, grain yield would be:

total shoot dry matter　=　[20x10^4 MJ/ha/day x 0.45 x 100 days x 2x10^{-6} t/MJ]

=　18 t/ha

and grain yield　=　18 t/ha x 0.40

=　7.2 t/ha

2.1.2 Grain yield as determined by water availability

A similar type of analysis can be carried out for water available to the crop (W; e.g., 750 mm), the proportion of the water transpired by that crop (P_{trans}; e.g., 0.60), water use efficiency of the transpired water (WUE; e.g., 0.040 t dry matter/mm), and HI (e.g., 0.40):

GY　=　[W x P_{trans} x WUE] x HI　　　　　[2]

=　[750 x 0.60 x 0.040] x 0.40

=　7.2 t/ha

2.1.3 Grain yield as determined by N availability

Again, the same analysis can be carried out for the nitrogen (N) available to the crop. NA is plant-available N (N as nitrate or ammonium) in the soil as available to the plant over the life-cycle of the crop (e.g., 300 kg N/ha). N_{uptake} is the fraction of available N in soil taken up by the plant (e.g., 0.50). NUE is nitrogen use efficiency (e.g., 0.12 t DM per kg N). HI is again harvest index (e.g., 0.40).

GY　=　[NA x N_{uptake} x NUE] x HI　　　　　[3]

=　[300 x 0.50 x 0.12] x 0.40

=　7.2 t/ha

2.1.4 Grain yield as determined by yield components

Grain yield itself can be divided into the components of plants per ha (e.g., 45,000/ha), ears per plant (EPP, e.g. 1.2), grains per ear (GPE, e.g. 400) and weight per grain (WPG, e.g. 334 mg, or 334 x 10^{-9} ton).

Thus: GY = Plants/ha x EPP x GPE x WPG [4]

= [45,000 x 1.2 x 400] x 334 x 10^{-9}

= [21,600,000] grains/ha x 334 x 10^{-9} tons per grain

= 7.2 t/ha

2.1.5 Grain yield as determined by *source* and *sink*

The question of whether maize yield is limited by plant characteristics relating to the supply of nutrients (*source*; e.g., nutrients, water, radiation, etc.) or the demand *(sink)* for assimilates, nutrients, water, radiation, etc., has been widely discussed. Depending on the environment, either the source or the sink can limit grain yield to varying degrees at almost any stage of development.

2.1.5.1 Grain yield as determined by source

The **total supply** of assimilates (or nutrients, or water) is determined by:

- The **amount of a growth factor** taken up by the plant, such as [RAD x %RI x GLD], [W x P_{trans}], or [NA x N_{uptake}].
- The **efficiency** with which that factor is converted by the plant into carbohydrates, proteins and lipids — the building blocks of the plant (e.g., RUE, WUE, NUE).
- The **time available for acquiring the growth factor**. This applies mainly to radiation, where the GLD term indicates the time the crop has available for radiation capture. If crop development is rapid, the time available for radiation capture is less than if crop development is slow. So in radiation limited situations, early maturing maize will yield less than late maturing maize. Under low N conditions, a considerable part of NA is supplied by mineralization, which proceeds at a rate determined by soil moisture, temperature, and the biological activity in the soil. Thus the **time** available to the crop to capture N released by mineralization will govern NA, and late maturing cultivars will therefore take up more N than early maturing cultivars.

Stress from drought or low N reduces leaf area (%RI), if the stress occurs before flowering. At any time of crop development, stress reduces crop photosynthesis rate (RUE, WUE or NUE in the other examples) and with that the total assimilates available to the crop. Stress after flowering reduces green leaf duration.

2.1.5.2 Grain yield as determined by sink

Grain yield is determined as well by the degree to which structures such as ears, kernels, and endosperm cells, which serve as repositories, or *sinks*, for assimilates, have been established. During the pre-flowering stage, maize establishes many more ears and florets than can finally be filled. In the two weeks bracketing flowering, the number of ears, kernels, and endosperm cells that are filled is determined. Maize is very sensitive to stress during this period. During grain filling, the supply of assimilates determines the extent to which ears, kernels, and endosperm cells established during flowering are filled.

2.1.5.3 Grain yield as determined by source or sink

The timing and intensity of stress determine the extent to which the *source* or the *sink* limits yield.

Example of sink limitation: Growth conditions are favorable during pre-flowering and a certain maize crop therefore establishes a large leaf area. There is stress during flowering time and therefore the crop can establish only few ears and kernels. After flowering the growing conditions may be favorable again, but the demand for assimilates by the kernels, and their capacity to absorb the available assimilate, will limit grain yield.

Example of source limitation: Growth conditions are favorable during pre-flowering and flowering, and a certain maize crop therefore establishes a large leaf area and many kernels and ears. Drought occurs after flowering causing the leaves to senesce early. The supply of assimilates will limit grain yield in this crop, and the plant will have many small kernels.

Because of the many ways stress can affect a maize crop, **genotype x environment interactions** are frequent. Imagine two maize genotypes, *A* and *B*. *A* has the ability to produce more ears and kernels when stress occurs at flowering. In the above example of sink limitation due to stress at flowering, *A* will yield more than *B*. In the above example of source limitation due to stress during grain-filling, both genotypes may yield equally, because the conditions are not such that the relative advantage of *A* can be expressed.

2.2 Water and the maize plant

Water is important to the plant as a solvent, as a cooling agent, as a reagent, and for maintaining structure by keeping the pressure inside cells high enough so that they are fully expanded (i.e., turgid). When the plant wilts, its turgor approaches zero, the cells begin to collapse, membranes suffer damage and proteins such as key enzymes can be denatured as their structure is altered. Cells can recover after drought stress. However the damage must be repaired, and this takes time (0.5 to 7 days). If damage is too great, the cells die.

2.2.1 Water potential, ψ

Plant and soil water potential is a measure of the pressure needed to extract free water from a plant or from soil. The symbol for water potential is usually given as the Greek letter, psi (ψ). The unit of water potential is the mega Pascal (MPa), though the older unit is the bar, equal to 1 atmospheric pressure. 1 MPa equals 10 bars.

Water potential and its components are usually given in negative terms, indicating the status of water compared with full saturation. Water moves from less negative to more negative water potentials. As the plant gets drier, the water potential term becomes more negative. Note that the water potential of air is around -80 MPa at 50% RH. Because water flows in direction of increasing negativity of ψ, there is almost always a tendency for water to evaporate from plant surfaces.

Water potential comprises three components:

$$\psi = \psi_p + \psi_s + \psi_m$$

where
ψ	=	water potential of the cell or soil
ψ_p	=	pressure potential
ψ_s	=	osmotic or solute potential
ψ_m	=	matrix potential

The matrix potential is ignored in most applications of Equation 5.

2.2.1.1 Water potential in the plant

Typical values for a leaf that is fully charged with water are: $\psi = 0$ MPa; $\psi_p = +1.4$ MPa (i.e., the cell is turgid, with a quite high internal pressure), and $\psi_s = -1.4$ MPa. Thus ψ_s and ψ_p balance each other and the water potential of the leaf is zero (Turner 1981).

When a leaf loses about 20% of its water content (relative water content = 0.80), there is a decrease in leaf water potential, turgor falls to zero, and solute potential becomes more negative as the cell contents become more concentrated. Under these circumstances we may find: $\psi = -1.6$ MPa, $\psi_p = 0$ (wilting), and $\psi_s = -1.6$ MPa. If a plant produces more solutes to enter the cell solution (osmotic adjustment), then ψ_p will increase as water is attracted to the cell vacuole and cytoplasm by osmosis, and the cells will again regain turgor, even though the overall leaf water potential stays constant.

2.2.1.2 Water potential in the soil

Water in the soil that is available to plants is between field capacity (soil water potential of -0.03 MPa) and permanent wilting point (soil water potential of -1.5 MPa). Clay holds about 200 mm water per m depth as available moisture; sand only 80 mm per m depth. About 55–65% of available water is contained between -0.03 and -0.5 MPa water potential and is easily available to the plant. The remainder of the available water is contained between -0.5 and -1.5 MPa and, even though a plant can extract that water, symptoms of wilting become visible. Soil texture and depth are crucial in determining water availability, W (Equation 2), to the crop.

2.2.2 Evapotranspiration, ET

Evapotranspiration is the term that describes the combination of evaporation (E) from soil and non-stomatal plant surfaces, and transpiration (T) from plant stomates. By far the largest proportion of water lost from the plant (>95%) is by transpiration.

2.2.2.1 Environmental factors affecting ET

- **Radiation**: The amount of radiation received by the crop area is the major force driving ET. When the sun is shining, ET will be high, and when conditions are cloudy it will be low. Radiation warms leaf surfaces and without the cooling evaporation of water, the leaf would overheat. In a closed canopy under well-watered conditions, about 85% of the energy arriving from the sun is dissipated by evaporation from crop surfaces and about 5% from evaporation from the soil. Only about 1% is used for photosynthesis. The remainder is dispersed by convectional air movements or by soil warming.

- **Temperature**: The relative humidity of the air falls as temperature rises and ψ_{air} becomes more negative. Therefore, maize grown in cool environments (e.g., the highlands) uses less water than maize growing in warm environments (e.g., the lowlands), even though their stomates may be open to the same extent. WUE is therefore higher in cool conditions than under hot conditions.

- **Relative humidity**: If ψ_{air} is more negative because the air is dry (e.g., blowing in over a desert), water usage will be high. Water usage will be least during rains, when ψ_{air} approaches zero.

- **Wind**: Crops use more water in windy weather. Wind removes damp air from above the crop that serves as a boundary layer, replacing it with drier air from surrounding areas. Thus ψ_{air} above the crop becomes more negative and evaporation is increased.

2.2.2.2 Plant factors affecting evapotranspiration

Loss of water from the leaf surface is an inescapable consequence of exchanging CO_2 during photosynthesis. Plants, like the lungs of humans, rely on gases entering solution before they can become part of the chemical reactions associated with photosynthesis. Therefore, a wet surface needs to be exposed to the atmosphere for exchanging CO_2. Transpiration and photosynthesis are thus closely linked (Tanner and Sinclair 1983), and crop water use and crop biomass production are closely associated. When the crop begins to run out of water or at night, stomates close and water use declines. This in turn prevents the exchange of CO_2 between plant and atmosphere and photosynthesis also stops.

- **Stomatal number and size** have relatively little effect on crop water usage until the stomates are virtually closed. This is because crop water usage depends on the overall gradient of ψ from the plant surface to the atmosphere, the *crop*-air boundary layer, rather than the boundary layer around any *individual leaf*. However, as stomates approach closure, the gradient of ψ_{leaf} to ψ_{air} becomes much steeper near the leaf surface and stomatal frequency and aperture begin to have a direct effect on crop water use.

- **Leaf area** affects water usage. Its main influence is on the evaporation/transpiration ratio (E/T). If radiation is not intercepted by the crop and strikes the ground, E/T will increase. A normal consequence is that weeds establish in those gaps in the crop canopy and E/T increases further as the weeds begin to use water for their own growth. Once complete crop cover has been established (radiation interception of > 95%, leaf area index > 3.5), further increases in leaf area have little effect on crop water usage.

2.2.3 Increasing W, P_{trans} and WUE (Equation 2)

To obtain high yields, the challenge is to pass as much water (W x P_{trans}) through the plant as "cheaply" (i.e., with a high WUE) as possible, and to maintain green assimilating leaf area as long as possible. Below are factors that influence each of the terms in Equation 2.

2.2.3.1 Plant available water, W

Plant available water, W, is affected by:

- **Rainfall and irrigation**, excluding losses through run-off.

- **Soil surface:** A crusted soil surface, or a bare soil that has no residue, can cause losses of 30-50% from heavy rainfall through runoff.

- **Soil depth:** Plants extract most water from the upper 70 cm of the soil because most of the root system is located there. Soils that are less than 70 cm deep because of rocks, compaction or soil acidity, may therefore reduce W.

- **Soil texture:** W is also affected by soil texture. Sand can hold 80 mm of plant-available water per m depth, whereas clay can store around 200 mm.

2.2.3.2 Proportion of the water transpired by the crop, P_{trans}

The proportion of the water transpired by the crop, P_{trans}, is affected by:

- **Roots:** Root length densities of 1.0-1.5 cm/cm^3 are needed to extract plant-available water from soil. Maize plants rarely achieve this below 70 cm, but values of 3-5 cm/cm^3 or more are common in the top 30 cm of soil. For better exploitation of available water, a better distribution of roots in the soil profile is preferable to partitioning more dry matter to roots.

- **Intercrops and weeds** use water, meaning that less is used by the maize crop.
- **Row-width, rapidity of cover, and senescence:** Whenever soil is exposed to sunlight, water evaporates from the surface, meaning less is passed through the maize crop.

2.2.3.3 Water use efficiency (WUE)

Water use efficiency (WUE) is equal to the ratio between assimilation and transpiration. It can be affected by:

$$WUE = (P_a - P_i) / (1.6 * (VP_i - VP_a)) \hspace{2cm} [6]$$

where
P_a = partial pressures of CO_2 in the air
P_i = partial pressures of CO_2 inside the leaf
VP_i = water vapor pressures inside the leaf
VP_a = water vapor pressures in the air

- **WUE is highest** when P_i is low (but this reduces plant growth), when the air is humid (VP_a high), when air temperatures are low (as in the highlands), and when other factors such as lack of nutrients or leaf disease and pests are not reducing growth.
- **Genetic variation for WUE** exists. It may be measured by the ratio of stable C isotopes, C^{13}/C^{12} (Δ) in the plant, which is proportional to P_i/P_a in C_3 plants, itself a reflection of the ratio of assimilatory capacity to stomatal conductance, and hence negatively associated with WUE (Hall et al. 1994). The same general relationship holds for C_4 plants but the level of discrimination of the isotopes is much lower.

2.2.4 Maize under drought stress

2.2.4.1 Drought stress affecting physiological traits at the cellular level

Drought stress affects some key physiological traits:

- **Abscisic acid** (ABA) accumulates. ABA is generated mainly in the roots, where it stimulates growth. It passes to leaves (and grain to a much lesser degree) where it causes leaf rolling, stomatal closure and accelerates leaf senescence. This happens even before hydraulic mechanisms reduce leaf turgor (Zhang et al. 1987). It seems likely that it is this "root signal" that causes the plant to reduce water loss. Thus, ABA is a plant growth regulator that helps the plant to *survive* drought stress but does not seem to contribute to *production* under drought. ABA passes as well to the grain, where it contributes to the abortion of tip grains during grain filling.
- Under mild to moderate stress, **cell expansion** is inhibited. This manifests itself in reduced leaf area expansion, followed by reduced silk growth, then reduced stem elongation, and finally reduced root growth, as stress intensifies.
- Under severe drought stress, **cell division** is inhibited, so even if the stress is alleviated the affected organs lack the cells for full expansion.
- **Osmotic adjustment**: Most species are able to form osmotically active substances in the cytoplasm and vacuole, in response to drought stress. This allows the plant to take up more soil water and maintains turgor and cell function for a longer time under drought. Osmotic adjustment is particularly apparent in sorghum, wheat and rice (the increase in negativity in ψ_s is from 1 to 1.7 MPa), but is much less in maize (0.3 to 0.5 MPa) (Bolaños and Edmeades 1991).
- **Accumulation of proline** has often been observed under severe drought. It may act as an osmolyte or protect protein structures, as turgor is lost.

- **Photo-oxidation of chlorophyll:** Drought affects Photosystem 2 more than Photosystem 1 in the photosynthetic mechanism. They become uncoupled, resulting in free, high-energy electrons in the leaf. Uncoupled electron transport leads to photo-oxidation of chlorophyll and loss of photosynthetic capacity. A very obvious bleaching of leaves exposed directly to the sun under drought stress can be observed.

- **Enzyme activity** is in general reduced under drought. For example, the conversion of sucrose to starch in the grain decreases because the activity of acid invertase—a key enzyme that converts sucrose to hexose sugars—diminishes (Westgate 1997; Zinselmeier et al. 1995).

2.2.4.2 Drought stress affecting the crop at the whole plant level

When the changes at the cellular level are summed at the whole plant level, we see the following responses to drought in maize:

- When drought ensues after initial rains, seeds germinate but the soil dries out, so that subsequent establishment and plant stand are badly affected.

- Drought leads to reduced leaf > silk > stem > root > grain expansion (in that order). Incomplete ground cover results from reduced leaf area expansion. Leaf senescence is accelerated (from the bottom of the plant first, but in conditions of high potential evapotranspiration it can also occur at the top of the plant as well), and this further reduces radiation interception.

- Stomatal closure occurs and photosynthesis and respiration decline from photo-oxidation and enzyme damage. Osmotic adjustment, especially in growing meristems, represent the plant's attempts to maintain cell division but does not seem to play a major role in maintaining growth when stress is severe.

- Assimilate fluxes to growing organs are reduced. Retarded silk growth gives rise to delayed silking and an increased anthesis-silking interval. Ear abortion and kernel abortion increase and plants may become barren. Barrenness can lead to a complete loss of grain yield. Female reproductive structures are more seriously damaged than tassels, though tassel blasting can occur if temperatures exceed 38°C.

- The root/shoot ratio increases slightly. When stress becomes more severe, root growth also decreases, and nutrient uptake by mass flow/diffusion in dry soil is sharply reduced.

- Remobilization of stem reserves can occur, when stress coincides with the phase of linear grain growth. In extreme cases this can result in premature lodging.

Summarizing, drought can affect maize production by decreasing plant stand during the seedling stage, by decreasing leaf area development and photosynthesis rate during the pre-flowering period, by decreasing ear and kernel set during the two weeks bracketing flowering, and by decreasing photosynthesis and inducing early leaf senescence during grain-filling. Additional reductions in production may come from an increased energy and nutrient consumption of drought adaptive responses, such as increased root growth under drought.

2.2.4.3 Drought and crop development

Drought affects maize grain yield to some degree at almost all growth stages, but the crop is the most susceptible during flowering (Fig. 2.1; Claassen and Shaw 1970; Denmead and Shaw 1960; Grant et al. 1989). Extreme sensitivity seems confined to the period -2 to 22 days after silking, with a peak at 7 days, and almost complete barrenness can occur if maize plants are stressed in the interval from just before tassel emergence to the beginning of grain fill (Grant et al. 1989).

Maize is thought to be more susceptible at flowering than other rainfed crops because its female

florets develop virtually at the same time and are usually borne on a single ear on a single stem. Unlike other cereals, in maize the male and female flowers are separated by as much as 1 m, and pollen and fragile stigmatic tissue are exposed to a dry and otherwise hostile atmosphere for pollination to occur. Furthermore and most importantly, silk growth and kernel number appear to depend directly on the flow of photosynthetic products during the three weeks of extreme sensitivity bracketing flowering (Schussler and Westgate 1995). When photosynthesis per plant at flowering is reduced by drought and several other abiotic stresses, silk growth is delayed, leading to an easily measured increase in the anthesis-silking interval (ASI), and kernel and ear abortion (Bolaños and Edmeades 1996; DuPlessis and Dijkhuis 1967; NeSmith and Ritchie 1992).
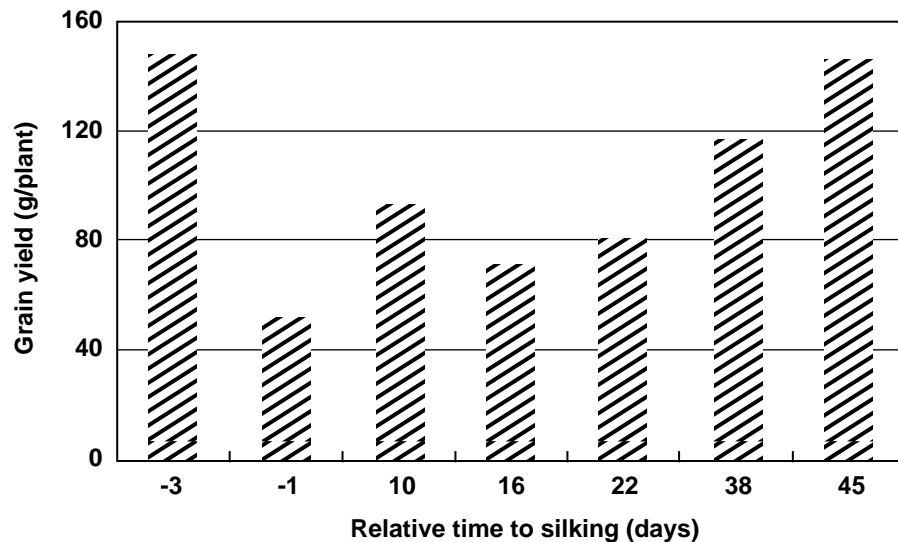
Fig. 2.1: Relationship between grain yield and timing of drought stress (Grant et al. 1989).

Although there are often reasonable quantities of plant reserves formed well before flowering and stored in the stem, the developing maize ear has very little capacity to mobilize and attract them in its first two weeks of life. Pollination may be successful in drought-stressed plants, only to be followed by abortion of the kernels a few days later (Westgate and Bassetti 1991; Westgate and Boyer 1986). Selection for reduced growth of stems (plant height) and tassel may reduce competition for assimilates at flowering and thereby decrease kernel abortion.

Once kernels enter the linear phase of biomass accumulation about 2-3 weeks after pollination, they develop the sink strength needed to attract reserve assimilates stored in the stem and husk. If kernels reach this stage they will normally grow to at least 30% of the weight of kernels on unstressed plants, even though drought may become much more severe (Bolaños and Edmeades 1996).

2.2.5 Breeding strategies for drought prone environments

2.2.5.1 Drought escape

Season length for maize under rainfed conditions is often defined as that time when precipitation is equal to or exceeds 50% of potential evapotranspiration, as determined by radiation, wind, and temperature. A major goal of breeding is to develop cultivars that can *escape* drought by being sufficiently early in maturity as to complete their life cycle within a given season length. In the lowland tropics, the lower limit of average seasonal rainfall for successful maize cultivation (> 1 t/ha) is around 400-500 mm; in midaltitude areas the minimum is about 350-450 mm; in the highlands it is around 300-400 mm. Because WUE is lower in the warmer lowlands, maize requires more rainfall than in the highlands.

Selection for earliness matches the **phenology** of the crop to the **pattern** of water availability. Since the time from sowing to flowering or physiological maturity is a highly heritable trait, selection for earliness can easily be accomplished. However, earliness carries a yield "penalty" when rainfall is higher than average. Under those circumstances, the yield of an early maturing cultivar is limited by the amount of radiation the cultivar can capture—normally less than that for a later maturing cultivar.

2.2.5.2 Drought tolerance

Precipitation is variable and cannot be predicted, especially in the tropics. No season is therefore "average" and a successful maize cultivar must be able to withstand some variation in rainfall from year to year. Drought tolerant cultivars are characterized by increased production under drought: mere survival with no grain yield is of little use. Except at seedling stage, traits that increase survival but not production are thus of little value in selection.

2.2.5.3 Selection for high yield potential

High yield potential (including heterosis) is a constitutive trait that often gives increased yield under moderate levels of drought; that is, when drought stress reduces yields by less than 50%. We can estimate the likelihood of spillovers from one environment to another by examining the genetic correlation for yields of the same cultivars grown in those two environments. Spillovers can be expected when the genetic correlation r_G between yields in stressed and well-watered sites is positive and significant. If r_G is weakly positive, zero or even negative, selection for yield potential alone does not affect drought tolerance much.

2.3 Nitrogen and the maize plant

Nitrogen is an essential component of all enzymes and therefore necessary for plant growth and development. It constitutes about one-sixth of the weight of proteins (many are enzymes), and is a basic element of nucleic acids. Nitrogen is especially plentiful in leaves, mainly in photosynthetic enzymes, where it may account for up to 4% of the dry weight. Because N uptake, biomass production, and grain yield are strongly correlated, the N requirement of a maize crop can be related to grain yield:

Grain yield (t/ha)	N required (kg/ha)
9.5	187
5.0	98
2.0	40

2.3.1 Increasing NA, N~uptake~ and NUE (Equation 3)

As with water in the case of drought, high grain yield under N stress is obtained by passing as much N (NA x N_{uptake}) through the plant as is "cheaply" (i.e., with a high NUE) possible, while maintaining active roots and green assimilating leaf area as long as possible.

2.3.1.1 N availability, NA

Most (95 to 99%) of the N in a field is not readily available to plants, because it is bound in the soil organic matter. Soil N is available to non-leguminous plants in the form of nitrate (NO_3) and ammonium (NH_4) ions. This pool of nitrate and ammonium is usually termed '**mineral N**'. Mineral N is increased by the mineralization of soil organic matter, by fertilization, by the release of ammonium ions from clay minerals, and slightly through rain. The pool of mineral N is decreased by plant uptake, by microbial immobilization, by leaching, by fixation of ammonium ions on clay minerals, and through gaseous losses. **Mineral N in the soil, integrated over the season and the root layer, determines the potential N available to the crop.**

The **amount of mineral N** in the soil can be measured. Soil samples should be taken within the main rooting zone of maize (i.e., to at least 60 cm depth). Samples should be immediately processed, frozen, or dried to prevent further microbial mineralization and immobilization after sampling.

Net N mineralization rate is affected by the amount and quality of the substrate (organic matter), clay type and content, soil temperature, soil water and nutrient content, and soil pH, but soil analyses are of limited usefulness here. Net mineralization rate in the field (*in situ*) is difficult to determine, because it is estimated as the difference between mineral N contents measured on two or more occasions and can vary significantly from one occasion to the next. Attempts have also been made at laboratory estimates (*ex situ*) of the mineralization potential of a soil. Such estimates may be more repeatable, but involve considerable modification of the factors that affect net N mineralization in the field. Lab studies can help identify soils that have a higher mineralization rate than others but cannot accurately predict the amount of N that will be mineralized over a certain season, unless soil-specific regressions have been performed. Despite the above, the following general rules do apply:

- High mineralization rates are associated with high clay content and high soil organic matter content (i.e., the amount of substrate), provided conditions for mineralization (soil temperature, soil water and nutrient content, soil pH) are favorable (Fig. 2.2).

- The higher the clay content, the longer it takes to deplete a field of mineral N (Fig. 2.2).

- N mineralization is greater from crop residues or organic fertilizer that have been *recently* added to the soil.

- N mineralization increases the mineral N pool in the soil when a field is left fallow.

- The higher the clay content, the less leaching of N.

Fig. 2.2. Nitrogen mineralization and soil organic matter content following the addition of organic matter (arrow) to two different soil types.

Time
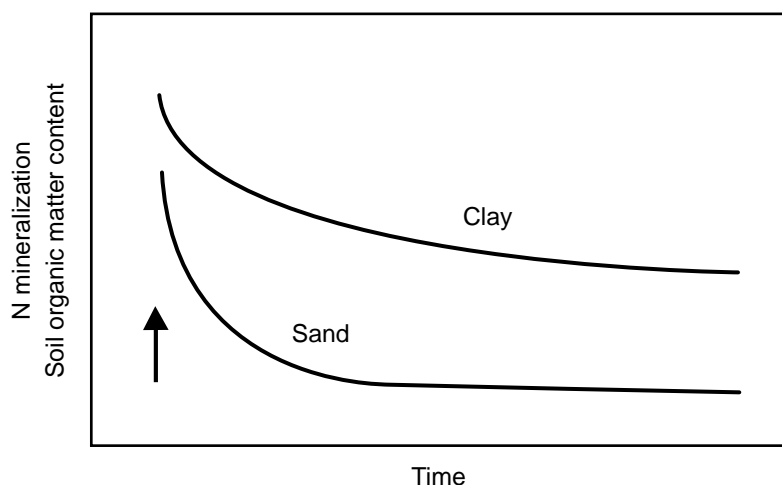
2.3.1.2 Proportion of the nitrogen taken up by the crop, N_{uptake}.

Recovery of available N by plants in the tropics is often only 35-50% and especially low in waterlogged soils. N_{uptake} is affected by:

- **Rooting depth**, since N can be leached below the effective rooting zone.

- **Root length density:** a root length density of around 1 cm/cm^3 is usually adequate for depleting the soil of plant-available N over a cropping period. Such a root length density is usually only found in the top 50 to 70 cm of the soil profile. Below this, some plant available N remains unused, and an increased root length density may increase N_{uptake}.

- **Duration of N uptake and assimilation:** At the beginning of the season mineral N supply in the soil usually exceeds the uptake capacity of maize. During the season, maize reduces the size of the mineral N pool because uptake (as much as 4-5 kg/ha per day) usually exceeds net N mineralization (that is, the difference between mineralization and immobilization; usually less than 1 kg/ha/day and, in N-depleted fields, less than 0.5 kg/ha/day). Maize can take up mineral N until about 4 to 6 weeks after flowering, if it is available in the rooting zone.

- As with water, non-leguminous **intercrops and weeds** reduce the N available to the crop.

2.3.1.3 Nitrogen use efficiency, NUE

NUE is affected by:

- **N supply:** NUE of absorbed N is around 30-70 kg grain per kg N at low levels of N availability. Hence a ratio of 20-40 kg grain/kg applied N at levels of applied N < 50 kg N/ha should be expected on highly N deficient soils with improved cultivars. There is a close correlation (r > 0.9) between grain yield and N uptake over a wide range of N availability. The relationship between N uptake and grain yield, however, is not linear, but rather a curve of diminishing returns to additional N inputs. Thus NUE decreases with increasing N input.

- **Other growth factors** (other nutrients, radiation, water, soil pH) may limit crop growth and NUE.

- **Genetic variation for NUE is large**. Stay-green is an important component of genetic variation in NUE, as a given amount of N in leaves can be used for photosynthesis and CO_2 assimilation over a longer time than in a plant where leaf senescence occurs earlier.

2.3.2 Maize under low N stress

2.3.2.1 Influence on crop photosynthesis

N stress reduces crop photosynthesis by reducing leaf area development and leaf photosynthesis rate and by accelerating leaf senescence. About 50% of all leaf N is directly involved in photosynthesis either as enzymes or as chlorophyll. Light-saturated photosynthetic rates show a strong dependence on leaf N content ($r > 0.75$), resulting in a curvilinear relationship between RUE and leaf N content that shows a saturation for maize at about 2% leaf N content. When N becomes scarce, plants reallocate N from older tissue (leaves, stalk) to younger tissue (leaves, grains), leading to early senescence of the older, lower leaf tissue.

2.3.2.2 Influence on root growth

Plants favor root growth over shoot growth under N stress and the root/shoot ratio increases. The absolute amount of roots, however, is usually less for plants grown under N stress than under normal N fertilization.

1.7.2.3 Influence on reproductive development

Relatively little is known about the effects of N stress on reproductive development. Initiation and development of reproductive structures occur in distinct phases, each of which can be affected by N stress. The number of potential kernel ovules is established early in plant development. The kernel row number is set by the time most tropical maize plants have 12-14 visible leaves and the number of kernels per row by the time 16-18 leaves are visible (Kiesselbach 1949). The number of ovules that ultimately develop into mature kernels is affected by the extent of kernel abortion in the two weeks bracketing flowering (Below 1997). Severe N stress delays both pollen shed and silking, but the delay in silking is relatively more, so that the ASI becomes greater under N stress at flowering. As with drought, silking delay is correlated with kernel and ear abortion.

2.3.2.4 N stress and crop development

Unlike drought, the pattern of N stress through the season is usually very similar from location to location. At the beginning of the season and especially with fertilizer applied, N supply usually exceeds crop demand. As the season progresses, N is taken up. Soil N mineralization is usually less than 1 kg N/ha/day, whereas a healthy maize crop can take up and assimilate 4 to 5 kg N/ha/day, leading to N depletion of the soil and N stress in the plant, as the season progresses. Plants adjust to some extent to N stress by remobilizing N from older tissue, a mechanism that does not affect yield in the case of tissue that contributes little to photosynthesis.

Depending on the timing of N stress in growing plant parts, different yield-determining factors are affected. Nitrogen stress before flowering reduces leaf area development, photosynthesis rate, and the number of ear spikelets (potential grains). Nitrogen stress during flowering stage results in kernel and ear abortion, whereas stress during grain-filling accelerates leaf senescence and reduces crop photosynthesis and kernel weight.

2.3.3 Breeding strategies for N stressed environments

There are few breeding programs that have deliberately tried to increase the low N tolerance of maize, and most selection for N stressed environments has been conducted under well-fertilized conditions. Most breeders are not aware that, as the severity of N stress under low N increases, the correlation between genotype performance under low N and well-fertilized conditions diminishes (Fig. 2.3). If yields in the target environment are less than 40 % of the yields obtained under well-fertilized conditions (as occurs in many tropical environments), germplasm should be evaluated under severe N stress as part of selection (Bänziger et al. 1997).
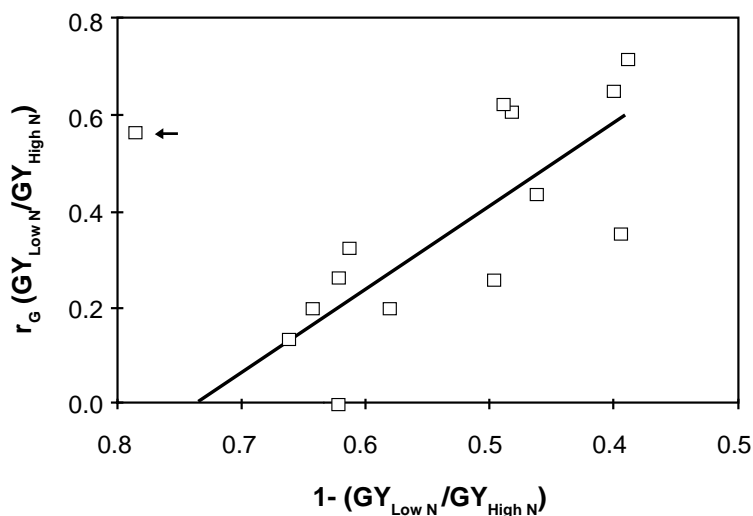
Fig. 2.3. Genetic correlation (r_G) between grain yields under low and high N vs. relative yield reduction under low N [1- ($GY_{Low N}/GY_{High N}$)] for 14 maize progeny trials evaluated at Poza Rica, México, between 1986 and 1995. Linear regression was y = 1.19 - 1.58x (R^2 = 0.62, P < 0.001, n = 13) with Exp. 11 excluded (from Bänziger et al. 1997).

2.4 Maize under drought and low N stress - Consequences for breeding

This short overview of the drought and low N stress physiology of maize shows that certain plant characteristics that are less relevant under non-stressed conditions become important for yield under drought and N stress. The most apparent example is the ability of a genotype to produce a grain-bearing ear under drought stress at flowering. This characteristic can only be observed under drought. Prolificacy under non-stress conditions (i.e., the ability to produce more than one fertile ear per main stem) is not closely related to the ability to produce an ear under drought (CIMMYT, unpublished data). If breeders do not evaluate germplasm under drought, they will likely not select genotypes that can produce an ear under drought.

The multitude of plant characteristics that could result in higher yields under stress is overwhelming, as is the number of genotype x environment interactions for grain yield that could be caused by genotypic variation in one of these characteristics. However, there are few single plant characteristics whose variation results in proven genotype x environment interactions for grain yield large enough to warrant their use in a targeted plant breeding

program (see Chapter 5). For breeding purposes, it is probably sufficient to **integrate plant characteristics over the main phases of development** (germination and establishment, pre-flowering period, flowering period, post-flowering period) and to ask whether

- The **susceptibility of grain yield** to stress in a given phase is sufficiently high.
- The **probability for stress** in that phase is sufficiently high in the target environment.
- The **probability of breeding success** in improving the stress tolerance of the plant in that phase is sufficiently high.
- Farmers can **compensate easily for loss** via other management practices, such as replanting.

Growth stages that have a high probability of being affected by stress and whose traits can be modified by breeding merit a focused effort to improve tolerance at that phase. "Focused" here means screening many genotypes for the target trait(s) (as defined in Chapter 1). Multilocation testing at advanced stages of breeding can serve to assess the stability of germplasm under other types of stress. Deciding on priority growth stages is essential, because it determines the type of stress management to be used and the secondary traits to be assessed.

Breeding for drought tolerance during flowering and/or post-flowering has the best chance of affecting maize production, provided those types of drought stress are relevant in the target environment. The apparent inconsistency of many data obtained under random drought stress stems from drought affecting maize at different growth stages. This is particulary evident when maize genotypes of differing maturity are included in the same trial: drought stress applied at a single moment in the trial may affect distinct growth stages in the different genotypes. If drought is consistently applied at the same growth stage, repeatable data are obtained.

In the case of **low N stress**, the breeding approach is simpler because the pattern of low N stress is very similar among low N fields: low N stress usually increases over time. Thus, **one relatively *severe* low N stress regime should be sufficient to assess low N stress tolerance** because, when combined with grain yield data from experiments under high N, it should allow prediction of genotype performance across a range of intermediate N levels (Fig. 2.3).

For **drought**, these considerations can be summarized as follows:

Stage	Susceptibility of yield to drought	Probability of drought stress	Probability of breeding success
Germination, establishment	High	Generally high	Low
Pre-flowering	Low	Random	Medium (?)
Flowering	High	Random	High
Post-flowering	Medium	Increasing towards end	Medium

3 Stress management

The key to breeding for both drought and low N tolerance is **to manage stress**. In the case of drought this is done by conducting experiments partly or entirely in the dry season and managing the stress through irrigation. In the case of low N, this is done by conducting experiments in fields that are depleted of N. The objective of such experiments is to measure the genotypic drought tolerance or to measure the genotypic low N tolerance. **The objective of such experiments is not to simulate a farmer's field, but to simulate a clearly defined stress that is relevant in farmers' fields.** If we select under random stresses, a combination of stresses or just 'low yields', we may well select for a different stress tolerance mechanism each time, and will likely not make much breeding progress.

Timing, intensity, and uniformity of the stress are factors to consider in stress management.

- **Timing** should be such that the growth stages targeted are susceptible to the stress, have a high probability of being affected by that stress in the target environment, and involve tolerance-related traits that can be modified through breeding.

- **Stress intensity** should be severe enough so that traits become important for yield distinct from those which affect yield under non-stressed conditions.

- **Uniformity:** If the stress is uniform over space and time, genetic differences will be easier to observe and progress will be greater.

3.1 Drought

3.1.1 Goal for drought stress applied at flowering stage

Irrigation is designed so that drought at flowering is severe enough to delay silking and cause ear abortion. The components that determine yield are number of ears and kernels per plant. Ideally, ASI should average about 4 to 8 days, ears per plant should average about 0.3 to 0.7, and yields should average around 1-2 t/ha (about 15-20% of well-watered yields). If drought stress at flowering is not severe enough, the accuracy (heritability and genetic variance) with which ASI and ears per plant can be measured decreases (Bolaños and Edmeades 1996).

3.1.2 Goal for drought stress applied during grain filling

Irrigation is designed so that drought develops directly after flowering and accelerates leaf senescence. The yield component affected in this case is kernel weight, because photosynthesis during grain-filling is reduced. Ideally, ASI should not be affected much by this type of a stress, but yields should be reduced to 50% of yield potential at least (i.e., if yields under unstressed conditions are around 7 t/ha, yields under this type of stress should range below 3.5 t/ha).

3.1.3 Managing drought stress through irrigation

3.1.3.1 Preparations before sowing drought stress experiments

The following considerations should be made before sowing drought stress experiments.

- Because it is difficult to divide a field into different stress regimes when sprinkler irrigation is used, **fields (drought blocks) should be managed with one stress level only,** and fields and/or stress levels should be far enough apart to prevent **border effects**.

- Irrigation has to be stopped earlier for early maturing germplasm than for late maturing germplasm, to obtain a similar drought stress intensity at a given growth stage. **Genotypes should therefore be grouped in experiments of similar maturity**.

- **Experiments should be grouped so that flowering time coincides for all experiments being subjected to a single stress treatment.** This can be done either by grouping experiments of different maturity in different fields and designing specific irrigation schedules for each field, or by planting early maturing experiments later so that flowering coincides with that of late maturing germplasm.

3.1.3.2 Irrigating drought experiments before the drought stress period

Before the period when drought stress is desired, irrigation intervals and other agronomic measures are designed so that the crop has optimal conditions for establishment and growth. The most important question when managing drought stress is: **When should irrigation be stopped so that drought stress is sufficiently intense at the critical growth stage (e.g., at flowering)?**

3.1.3.3 Using a crop water balance for determining the date of the last irrigation

A crop water balance is usually used to calculate irrigation intervals for crops that should be irrigated when they show first symptoms of drought stress. It takes about twice this interval to obtain a stress level that reduces maize yields to the extent needed for drought experiments.

To calculate a **crop water balance for severe drought stress at flowering**, proceed as follows:

1. **Estimate average anthesis date (AD) for your trials:** Be aware that the temperature during the trial determines crop development. If the temperature between planting and flowering in your drought trials is lower than during your usual main season, it will take longer for the crop to reach flowering. If the temperature between planting and flowering in your drought trials is higher than your usual main season temperature, it will take less time for the crop to reach flowering. Calculating the temperature sum (heat units) between planting and flowering can help to determine anthesis date. The temperature sum between planting and flowering is constant for a certain maturity group, provided photoperiod effects can be disregarded. It can be calculated as (Kiniry 1991):

$$\text{Temperature sum} = \sum((T_{max}+T_{min})/2-8) \qquad [7]$$

$$\text{where } T_{max} \quad = \quad \text{daily maximum temperature}$$
$$\text{if } T_{max} > 34 \text{ then } T_{max} = 34- 2.6*(T_{max}-34)$$
$$\text{if } T_{max} > 44 \text{ then } T_{max} = 34- 2.6*(44-34) = 8$$
$$T_{min} \quad = \quad \text{daily minimum temperature}$$
$$\text{if } T_{min} < 8 \text{ then } T_{min} = 8$$
$$\sum \quad = \quad \text{make the sum for the period planting to anthesis}$$

2. **Estimate daily water consumption:** There are various methods for estimating daily water consumption of crops (Doorenbos et al. 1984). They differ in the type of weather data they use. Most experiment stations measure pan evaporation (water evaporation from an open water surface). Daily water consumption (DWC) of maize can be calculated as:

$$DWC = PE * K_p * K_c \qquad\qquad [8]$$

where PE   =   pan evaporation, as measured in a Class A pan in a standard meteorological station installation

K_p =   pan coefficient (determine from Table 3.1)

K_c =   crop coefficient

Crop coefficient, K_c: Maize has a K_c of about 0.25 at germination, 0.50 at 6-leaf stage, 1.10 at flowering stage, and 0.40 near maturity. We suggest that you use an average K_c of about 0.80 for calculating daily water consumption near flowering. Note that the crop coefficient is influenced by leaf area, stomata opening, and the relative importance of transpiration (from plants) and evaporation (from the soil). Inbred materials have a smaller crop coefficient than full vigor materials, because they have less leaf area. Stressed plants have a smaller crop coefficient than unstressed plants, because they close their stomata. Plants that were stressed during early growth stages have a smaller crop coefficient at later growth stages because their leaf area is reduced.

3. **Determine soil texture:** from Table 3.2.

4. **Determine plant-available water (PAW, in mm/10cm depth):** from Table 3.3.

5. **Estimate the rooting depth (RD) of maize:** The rooting depth of maize is about 10 cm at germination, 30 cm at the 6-leaf stage, and 70–100 cm at flowering, depending on how porous or compacted the soil. Inbred materials generally have fewer roots and shallower root development than full vigor materials.

6. **Estimate the amount of water available (W) to the crop until first stress symptoms are visible:** Maize shows first symptoms of stress when 55 to 65% of [PAW * RD] is used, i.e.

$$W = RD/10 * PAW * 0.65 \qquad\qquad [9]$$

7. **Calculate the time (T_1) until maize shows <u>first</u> symptoms of drought stress:**

$$T_1 = WA/DWC \qquad\qquad [10]$$

8. **Calculate the time of the last irrigation (T_2):**

$$T_2 = AD - 2 * T_1$$

Note: it takes about twice as long to obtain severe drought stress (desirable in a drought experiment) as first visible drought symptoms. Therefore T_1 is multiplied by 2.

Calculation of a crop water balance can allow quantitative insights on factors that affect the length of time after the last irrigation or rainfall until first stress symptoms. This operation will also point up the limits of any predictions: factors that are difficult to determine—such as effective rooting depth—can modify the water balance considerably.

Table 3.1. Pan coefficient, K_p, for a Class A pan in a standard meteorological station installation. Distance refers to the 'fetch', or the distance wind passes over the crop or fallow before it reaches the pan.

Wind	Distance of crop	Relative humidity (%)		Distance of fallow	Relative humidity (%)		
(km/day)	(m)	< 40	40-70	> 70	(m)	< 40	40-70	> 70
175	1	0.55	0.65	0.75	1	0.70	0.80	0.85
Light	10	0.65	0.75	0.85	10	0.60	0.70	0.80
	100	0.70	0.80	0.85	100	0.55	0.65	0.75
	1000	0.75	0.85	0.85	1000	0.50	0.60	0.70
175-425	1	0.50	0.60	0.65	1	0.65	0.75	0.80
Moderate	10	0.60	0.70	0.75	10	0.55	0.65	0.70
	100	0.65	0.75	0.80	100	0.50	0.60	0.65
	1000	0.70	0.80	0.80	1000	0.45	0.55	0.60
425-700	1	0.45	0.50	0.60	1	0.60	0.65	0.70
Strong	10	0.55	0.60	0.65	10	0.50	0.55	0.65
	100	0.60	0.65	0.70	100	0.45	0.50	0.60
	1000	0.65	0.70	0.75	1000	0.40	0.45	0.55
> 700	1	0.40	0.45	0.50	1	0.50	0.60	0.65
Very	10	0.45	0.55	0.60	10	0.45	0.50	0.55
strong	100	0.50	0.60	0.65	100	0.40	0.45	0.50
	1000	0.55	0.60	0.65	1000	0.35	0.40	0.45

Table 3.2 Determining soil texture: 1. From a ball of about 3 cm diameter from fine soil; 2. Drip water onto the soil until starts sticking to the hand.

Type	Description
Sand	The soil remains loose. You cannot form a ball.
Sand loam	The soil can be rolled into a short thick cylinder.
Loam	The soil can be rolled in a 15 cm cylinder that breaks when bent.
Clay loam	As loam, but the soil can be bent into a U.
Light clay	As loam, but the soil can be bent into a circle that shows cracks.
Heavy clay	As loam, but the soil can be bent into a circle without showing cracks.

Table 3.3. Characteristics of various soils.

	Field capacity	Permanent wilting point	Plant-available water	Bulk density
	(Vol %)	(Vol %)	(mm/10 cm depth)	(g/cm^3)
Sand	15	7	8 (6 -10)	1.65
Sandy loam	21	9	12 (9 - 15)	1.50
Loam	31	14	17 (14 - 20)	1.40
Clay loam	36	17	19 (16 - 22)	1.35
Light clay	40	19	21 (18 - 23)	1.30
Heavy clay	44	21	23 (20 - 25)	1.25

3.1.3.4 Using a crop simulation model for determining the date of the last irrigation

Crop simulation models provide a more sophisticated estimate of the crop water balance than the method described above. If they are to be used for managing irrigation in drought experiments, they still rely on an accurate calibration based on site- and crop-specific data, especially with respect to water conditions at various depths in the soil at the start of the simulation period.

Note that the timing for stopping irrigation can never be precisely determined. This is because evaporation between the time when irrigation stops and the growth stage when stress should occur is a prediction based on previous years' weather data. The actual conditions for a given season may differ significantly from the long-term average. **Note: Breeders usually underestimate the time it takes to develop severe stress in a maize crop,** because they take usual irrigation intervals or the time it takes to first visible drought symptoms as guidelines for terminating irrigation. However, it will take considerably longer than the time to first visible drought symptoms to produce a severely drought stressed maize crop.

3.1.3.5 Using an experiment for determining the date of the last irrigation

An experiment where seed of a particular maize genotype is sown at different dates but irrigated at the same time can help to improve drought stress management in following years. Plant 10 rows of maize 5 times at 5-day intervals (that is, a total of 50 rows of maize in 5 sections with 5 different planting dates). Irrigate all on the *same day*, and apply the last irrigation before flowering when you predict that it will result in ideal drought stress intensity for the 2nd planting date. The first rows sown should exhibit less stress than maize from the 2nd planting date, because the last irrigation is applied relatively later in crop development. By the same token, all rows planted from the 3rd date on should experience greater stress. Determine the planting date for which stress intensity was ideal and calculate the time between the last irrigation before flowering and flowering. Use this time period for scheduling the last irrigation for stress experiments in coming years.

3.1.3.6 Using two different drought stress levels

The problem of estimating the time when irrigation should be stopped may as well be solved by managing **two drought stress levels**, each in a different field where sets of the same trials are planted. The two stress levels create selection environments that are representative of two different, important types of drought stress: flowering stress and grain-filling stress. In both cases, optimal irrigation at regular intervals is applied for germination and crop establishment, until the last irrigation before the stress period.

Severe stress: Irrigation is timed so that severe drought stress is predicted for flowering. An additional irrigation is applied about 14 days after the end of male flowering to ensure that the small amount of grain formed will fill adequately.

Intermediate stress: This treatment receives one irrigation more before flowering than the severe stress treatment, but no further irrigation after flowering or during grain filling. This stress regime targets grain-filling.

If both experiments are planted at the same time, they provide the following **management options**:

- If evapotranspiration and crop development proceed as predicted, the severe stress treatment results in flowering drought stress and the intermediate treatment results in severe grain filling stress.

- If evapotranspiration is greater or crop development slower than expected, the intermediate stress treatment will result in drought stress at flowering. The severe stress treatment can be rescued with an irrigation near flowering when stress becomes too severe; it thus becomes a grain-filling stress treatment.

- If evapotranspiration is much lower or crop development faster than predicted, the severe and intermediate stress treatment will result in two levels of grain filling stress, and there will be no treatment with drought stress at flowering.

3.1.3.7 Application of irrigation after flowering stress

After drought stress at flowering, an additional irrigation may be necessary to ensure grain filling. The following guidelines can help.

- If the average ASI of the drought stress block is less than 3 days, do not apply any further irrigations after flowering.

- If the average ASI of the drought stress block is between 3 to 5 days, apply one irrigation two weeks after male flowering is completed.

- If the average ASI of the drought stress block is between 5 to 8 days, apply one irrigation one week after male flowering is completed.

- If the average ASI of the drought stress block is estimated at more than 8 days, apply irrigation when 80-100% of the plots have completed male flowering.

Note: Irrigation should be applied only *before* silking starts or *after* male flowering is complete; not during flowering, when the susceptibility of maize changes rapidly.

3.1.4 Improving the uniformity of drought stress

Variation in drought stress intensity comes from two sources: variation in soil characteristics and variation in the application of irrigation. Variation in soil characteristics is almost impossible to correct, unless you move to another field. Variation in the application of irrigation can and should be corrected.

Normal experimental precision is required for irrigation and crop management until the last irrigation before the stress period is due to begin. **It is vitally important that the last irrigation before the stress period begins is applied as uniformly as possible.** To achieve this:

- Choose a field that is as level as possible for drought experiments. Try to avoid old river beds, or areas where soil depth or texture is known to vary over short distances.
- If using sprinkler irrigation, apply it when there is no wind. Note that wind usually varies over the day and you should choose a time of the day when there is little or no wind.
- Make sure that risers are high enough so that water jets do not damage plants near the sprinklers.
- Make sure *beforehand* that the irrigation system is set up properly, that pipe connections are sealed and that sprinkler heads are clean and work properly; replace sprinkler heads that do not work properly; if necessary exchange nozzles.
- When the irrigation system is turned on, remove the end cap of the main pipe for a brief period to flush out dirt that may clog sprinkler heads.
- Apply irrigation so that, as a minimum, field capacity in all parts of the field is reached; where more water is applied than necessary for reaching field capacity, the water will drain, but the whole field will be at field capacity for one or two days after irrigation, thus leveling differences that might have resulted from non-uniform irrigation.
- Use carefully leveled catch cans to measure the amount of irrigation at places in the field where irrigation is expected to be the lowest. If placed systematically in the field, the volume of water collected in the catch cans can be used to adjust the sprinklers for uniformity.

Increased uniformity of water application before stress onset will translate into more uniform drought stress, more uniform plant performance, and increased breeding progress.

3.1.5 Analysis of drought experiments

Once irrigation is stopped, drought stress increases over time. Later maturing germplasm will be more stressed and therefore lower yielding than early maturing germplasm. A systematic increase in stress intensity with time and a systematic yield decrease with later anthesis dates can be accounted for in data analyses; not so for non-systematic changes in stress intensity, such as an irrigation application or rainfall event during flowering.

3.2 Low N stress

3.2.1. Goal

Ideally, managed low N stress should result in yield levels that are about 25 to 35% of those obtained under well-fertilized conditions at a given site. Thus, if the yield at a site under full fertilization is around 7 t/ha, an optimal level of low N stress should result in yields of 1.5 to 2.5 t/ha. Under such intense stress, plant traits that affect yield are different from those relating to yield under non-stress conditions, so genetic variation for low N tolerance can be observed (Bänziger et al. 1997). If yields under low N stress are greater than 50% of those obtained under well-fertilized conditions, they are related more to genotypic yield potential than to mechanisms that impart tolerance to low N stress, and N stress tolerant genotypes cannot be easily discriminated.

3.1.2 Managing low N stress

3.1.2.1 Amount of mineral N at the beginning of the season

The relationship between N uptake and grain yield is curvilinear. Thus, yields at 25 to 35% of those for well-fertilized conditions represent an update of no more than 20 to 25% of the N uptake for maize under well-fertilized conditions. So, if N uptake under well-fertilized conditions is 200 kg N per ha, N uptake under low N stressed conditions should not be more than 40 to 50 kg N per ha. If N uptake under well-fertilized conditions is only 100 kg N per ha because of other limiting growth factors such as drought, N uptake under low N stressed conditions should be between 20 to 25 kg N per ha only. Considering that mineral N will be produced throughout the season the above provides some indication of desirable soil mineral N content for the beginning of the season, in low N selection trials (and it turns out that the desirable levels are quite low). Assuming that no N fertilizer is applied and that ammonium exchange with clay minerals is negligible, then **a small amount of mineral N in the soil at the beginning of the season and a slow net mineralization rate during the season will result in the rapid development of intense N stress.** Large amounts of mineral N in the soil and a faster rate of mineralization will result in little or no N stress.

3.1.2.2 Using the same field over several seasons

Because of the difficulties of estimating N availability through soil (or plant) analyses, it is advantageous to use the same low N field over several seasons, in essence capitalizing on the results obtained with the N stress of the past season to manage the N stress of the following season. Nitrogen stress intensity can be increased by:

- Choosing a field with a sandy soil texture, but where no factors (other nutrients, water, soil pH) other than N limit crop growth.
- Continuously using the same low N field (Fig. 2.2).
- Not applying any N fertilizer, either in chemical or organic form.
- Reducing the length of fallow between the previous crop and the planting date of maize.
- Growing non-leguminous crops with a high biomass production in the previous season and removing that biomass: the higher the biomass production, the more N is removed from the soil.

- Removing or burning the stover of the previous crop directly after harvest. If the stover is not immediately removed, some of the organic matter starts to decay and N is returned to the soil.

- Increasing the sowing density of maize. If plant density is high, N supply per plant is less, and N stress intensity *per plant* develops more rapidly and is more severe. Grain yield per unit area may be the same when two different plant densities are compared in a low N field, but N stress intensity per plant is higher when the density is high. Because we are interested in modifying plant characteristics that are important determinants of yield under N stress, increasing the plant density is a desirable and useful strategy to the extent that N and not light remains the limiting environmental factor for growth.

- Planting a non-leguminous intercrop with maize; the more biomass the intercrop produces the more N it removes, and the greater its effect. The sowing density of maize should not be decreased when an intercrop is used. The intercrop should be uniformly established and not compete with maize for light.

3.1.2.3 Need for applying N fertilizer in a low N experiment

N fertilizer should only be applied in low N experiments if yields are expected to fall below 20% of yields measured under well-fertilized conditions at that location. If N fertilizer needs to be applied, no more than 20 kg N/ha should be applied at planting, and an additional dose should only be given if plant development indicates that yields will likely fall below 20% of well-fertilized yields.

3.2.3 Improving the uniformity of low N stress

Variation in soil N supply due to inherent differences in soil characteristics poses one of the greatest problems in breeding for low N tolerance. Such variation is often masked by N fertilization and is thus difficult to assess in well-fertilized fields. When such fields are depleted of N, inherent spatial variation in soil fertility becomes apparent. These differences are almost impossible to correct; unless statistical tools can adjust for field variation, it is better to abandon such a field.

Uniform soil texture and uniform soil depth are the most important points to consider when judging the uniformity of a field. Soil texture is related to soil organic matter and therefore to N mineralization. Soil texture also affects the speed and quantity of leaching of N. Soil depth is one of the factors determining the amount of mineral N available to the crop (see above) and mineral N supply often varies to a similar extent as the soil depth.

It is always important to manage fields on experiment stations as uniformly as possible, but this is even more important for low N fields. **Crop management practices that supply or remove different amounts of N in a non-uniform manner to/from various parts of the field should be prevented.**

- A low N field needs to be planted entirely with no free rows, both when cropped with maize or other crops. If intercrops are established, they need to have a uniform stand and be sown over the entire block.

- If there are alleys between plots, they need to be at the same place every season.

- If crop stover is removed, it needs to be removed entirely and at the same time.

4 Statistical designs and layout of experiments

Differences between genotypes are usually smaller under stress conditions, and superior genotypes are therefore more difficult to detect (Rosielle and Hamblin 1981). As a consequence, heritability of grain yield decreases.

Breeders have traditionally coped with this problem by evaluating genotypes mainly under high yielding conditions, where differences between genotypes, and therefore heritability, for grain yield are larger, ignoring the fact that superior genotypes under high yielding conditions are not necessarily high yielding under stress conditions. In the CIMMYT low N breeding program, we calculated that breeding progress for target environments where N stress decreases yields by more than 40% is higher if we use a low N selection environment than if we use a high N selection environment. Thus, in spite of the lower heritability under low N, we can make more breeding progress selecting under low N for a low N target environment because genotypes we identify to be superior under high N do not correspond with genotypes that are superior under low N.

Given the need to select under stress, it is a challenge to keep the heritability for yield as high as possible. Since genetic variance under stress cannot be changed, every measure should be used to keep experimental error low.

The experimental error variance can be reduced by:

- Using uniform fields and managing them uniformly (discussed above).
- Increasing the number of replicates in an experiment.
- Ensuring that the experiment is bordered uniformly and adequately towards alleys in the field and field borders.
- Using improved statistical designs that partly control the variation within a replicate.
- Choosing an optimal field layout that reduces the variation within replicates.
- Using statistical analysis tools that consider spatial variation.

Note that these principles apply for both stressed and non-stressed experiments, but that the relative importance of reducing error variance is larger under stress.

4.1 Increasing the number of replicates

Increasing the number of replicates does not necessarily increase the efficiency of a breeding program, because more resources (land, seed, etc.) have to be used or fewer genotypes can be evaluated. **Increasing the number of replicates and decreasing the plot size at the same time** may increase selection efficiency as long as the total number of plants sampled per genotype does not decrease much. The number of plants sampled for a genotype using smaller plots but more replicates usually does decrease, because plants next to alleys need to be removed in stress experiments where border effects can be extremely large. Decreasing plot size results in larger border effects from the neighboring plots. However, the trade-off of increased border effects between neighboring plots is apparently not as large as the relative gain from increasing the number of replicates (Bänziger et al. 1995; Castleberry 1986). Under stress, field variation is often small-to medium-scale and a smaller plot size allows an entire experiment, replicate, or incomplete block to be fitted into a more uniform area (see below).

Border effect: Stress experiments need to be well-bordered towards the edge of the field or towards a free row, as more of the limiting growth factor is available to border rows.

Stress management: Maize field on clay soil, at the time when last irrigation before flowering is to be applied to achieve flowering drought stress.

Stress management: Drought stress induces leaf senescence by flowering time.

Stress management: Left, a well-fertilized field; right, an N stressed field

Stress management: Severely drought-stressed maize during grain-filling.

Border effect: The first one to two plants next to an alley have more of the limiting growth factor available than the plants within the row and are less stressed.

Border effect: From left for right, ears from the first (next to the alley), second, and third plant of an N stressed plot. The plant next to the alley was less stressed. For assessing plot yields under stress, ears of the first plant next to the alley should be discarded.

33

Leaf rolling under drought. Photos show leaf rolling scores of 1, 2, 3, 4, 5.

Large and small tassel size.

Leaf senescence under drought. Photos show leaf senescence scores of 2, 4, 6, 7, 9.

Leaf firing under drought, an undesirable trait.

Tassel blast under drought, an undesirable trait.

Leaf senescence under low N. Photos show leaf senescence scores of 1, 3, 5, 7, 9.

Grain yield and number of ears per plant are important characteristics, when screening for drought or low N tolerance. Both ear groups were harvested from the same number of plants and the same plot size, but the genotype on the right had more barren plants.

Increasing the number of replicates and decreasing plot size is advantageous in advanced yield trials that usually have fewer entries but large plots. For example, a yield trial that is planted with four-row plots and three replicates under non-stress conditions could be planted with six replicates and two-row plots under drought and low N, and more precise information on genotype performance would be obtained, in most cases.

4.2 Improved statistical designs

4.2.1 Unreplicated experiments

Improved designs are available both for replicated and unreplicated experiments. In the case of unreplicated experiments, checks planted systematically over the field allow the breeder to discriminate between genotype performance and field variation (augmented designs). In general, some 20% of the plot area is planted to one or several check entries.

Example: An augmented design may allow a breeder to compare 496 genotypes and 124 check plots in 62 sub-blocks of 10, where 8 plots are genotypes and 2 plots are checks. There are 4 check genotypes, each repeated 31 times. In this same area the breeder could evaluate only 310 genotypes in two replicates.

Augmented designs are very valuable during prescreening involving many genotypes.

4.2.2 Replicated experiments

In the case of replicated experiments, improved statistical designs are available that provide better control of within-replicate variation, and hence experimental error, than randomized complete block designs (RCBD). Improved designs adjust genotype means for variation occurring within a replicate, with the result that genotype means are no longer equal to the arithmetic average of individual plot data. Improved designs provide a better estimate of the true genotype means. Computer software is available that creates and analyzes improved designs in a routine manner. **By using improved, replicated statistical designs instead of an RCBD, a breeder can increase breeding progress without additional costs**.

We discuss here only two of several improved designs available: lattice designs and covariate analysis.

4.2.2.1 Lattice designs

Lattice designs group genotypes in incomplete blocks within each replicate and adjust genotype means for incomplete block effects; i.e., soil variation among incomplete blocks within a replicate. Compared to other lattice designs, **alpha lattice design**s (an unbalanced type of lattice design) pose very few restrictions on numbers of treatments, replicates, incomplete blocks, or spatial layout. Block size is determined by the scientist. When soil variation is low, lattice designs with an incomplete block size equal to or slightly smaller than the square root of the treatment number are the most efficient. For example, with 240 treatments, a design of 16 blocks with 15 plots per block would be appropriate when soil variation is low. When soil variation is high, incomplete lattices with a smaller block size are more effective. In the case of 240 treatments, 24 blocks with 10 plots per block or 30 blocks with 8 plots per block would be suitable when soil variation is high. In the CIMMYT low N breeding program, use of such lattice designs increased breeding progress by 20% on average (Bänziger and Lafitte 1997b). Simple software to make and analize alpha lattice designs is available from CIMMYT.

4.2.2.2 Covariate analysis

Because much of the variation in stress trials is due to inherent differences in soil characteristics, variation consistently shows up at the same place within the field over several seasons. Plot yields of a single maize check sown among trial genotypes at a frequency from 1:1 to 1:5 and measured in only one season could be used effectively as a covariate in the analysis of variance for several following seasons. Lafitte et al. (1997) showed that such a **covariate adjustment can reduce error variance to a greater extent than lattice designs, when the number of genotypes evaluated is low and plot size is large (e.g., in advanced yield trials)**.

Several new designs under development hold even greater promise for controlling error variance. Among these are row and column designs and other types of spatial adjustments in two dimensions. All require the "geographical" (row, column) coordinates of each plot to be entered along with the data.

4.3 Field layout

Experiments should be laid out in the field so that:

- Replicates and incomplete blocks are as compact (square) as possible.
- Replicates are arranged in a manner that they lay at right angles to trends in stress intensity.
- Entire experiments, replicates or incomplete blocks are placed within areas of uniform stress.

4.3.1 Statistical analysis tools that consider spatial variation

Computer software packages such as ASREML (Gilmour et al. 1998) take into account spatial variability in field trials and may therefore reduce error variance and improve estimates of variety means even within replicates and incomplete blocks.

4.4 Border effects from alleys

Plants grown next to an alley have greater access to the factor (water, N) that is causing stress in the rest of the plot since their roots penetrate the soil of the alley where there is no competition for the available N and water. They are therefore less stressed. In a severely stressed drought trial, the plant next to the alley can produce up to half of the entire plot yield. **Plants bordering alleys need to be removed before harvest. They are far less stressed, and so may disproportionately affect plot yield while not representing the mean performance of that genotype accurately**.

5 Secondary traits

5.1 Why use secondary traits?

Breeders' primary interest is in grain yield. So why do we need other, secondary traits to assess drought and low N tolerance in maize? In a drought and low N breeding program, secondary traits are valuable for the following reasons.

- They can **improve the precision with which drought or low N tolerant genotypes are identified**, compared to measuring only grain yield under drought or low N stress. This is because under stress the heritability of grain yield usually decreases, whereas the heritability of some secondary traits remains high, while at the same time the genetic correlation between grain yield and those traits increases sharply (Bänziger and Lafitte 1997a; Bolaños and Edmeades 1996).

- They can **demonstrate the degree to which a crop was stressed by drought or low N**.

- If observed before or at flowering, they **can be used for selecting desirable crossing parents. Under this scenario, a crossing block is planted separately and usually slightly delayed to the drought or low N trial and data from the trial are used to select the crossing parents.**

- If observed before maturity, they **can be used for preliminary selection** when turn-around time between seasons is short.

Most breeders use secondary traits. A breeder who is interested in disease resistance is not just measuring yield under disease pressure, but assesses disease incidence as well. Many breeders consciously or unconsciously use an ideotype approach (an ideotype can be defined as the target plant the breeder has in mind when selecting). Selection for stay-green, upright leaves, small tassels, dark green leaves, etc., is common, although the additional improvement in genetic progress such traits provide over selecting for grain yield alone has rarely been measured objectively.

CIMMYT physiologists have evaluated many secondary traits for their value in a drought or low N breeding program. The following recommendations on the use of secondary traits are based on the results (Bänziger and Lafitte 1997a; Bolaños and Edmeades 1993a; 1993b; Bolaños et al. 1993; Edmeades et al. 1993; Lafitte and Edmeades 1994a; 1994b; 1994c). Under low N, we estimated that selection gains were increased 20% through use of secondary traits.

5.2 How do we decide on the value of secondary traits in a drought or low N breeding program?

Many reviews on plant characteristics related to drought or low N tolerance have been written (e.g. Hsiao 1973; Ludlow and Muchow 1990; Turner 1986), but few secondary traits have been used in breeding programs and even fewer have proven to contribute to the improvement of drought or low N tolerance in maize.

Edmeades et al. (1998) establishied that an ideal secondary trait should be:

- Genetically associated with grain yield under stress.
- Highly heritable.
- Genetically variable.
- Cheap and fast to measure.
- Stable within the measurement period.
- Not associated with a yield penalty under unstressed conditions.
- Observed at or before flowering, so that undesirable parents are not crossed.
- A reliable estimator of yield potential before final harvest.

Many recommendations on the use of secondary traits have been made to breeders, based on phenotypic correlations between such traits and grain yield. Unfortunately, many such correlations have been calculated from few varieties where outlying values may greatly affect the sign and magnitude of the correlation. In addition, **for a breeder it is not sufficient to know that a secondary trait is related to drought or low N tolerance. Rather, it is important to know that breeding progress using grain yield and a given secondary trait in selection is greater than progress using grain yield alone.** Thus, not only must secondary traits be *identified*, but their *value* in breeding must be proven. This can be accomplished using:

- Analyses of genetic correlations and heritability among progenies of a single population.
- Selection indices (Fukai and Cooper 1995).
- Divergent selection that creates synthetics or near-isogenic lines that have a similar genetic background but differ for a single, selected trait. Correlated response of grain yield can then be measured.
- Analyzing physiological and morphological changes in varieties that have been consistently selected for performance under drought or low N stress.
- Simulation models.

5.3 Secondary traits that help to identify drought tolerance

The following traits are recommended for use in a drought breeding program. They are listed in order of decreasing importance.

5.3.1 Grain yield

- Heritability: medium under grain filling stress, medium to low under flowering stress.
- Relationship with grain yield: high.
- Selection: for increased grain yield.
- Stress type: to be measured under flowering or grain filling drought stress.
- Measurement: shelled, adjusted for grain moisture.

Remarks: Shelling percentage varies considerably under drought. Grain weight, not ear weight, should be used for calculating grain yield.

5.3.2   Ears per plant

- Heritability: high and increasing with stress intensity.
- Relationship with grain yield: high under flowering stress.
- Selection: for more ears per plant (i.e., less barrenness).
- Stress type: to be measured under flowering drought stress; heritability and genetic variance is largest when flowering stress is intense enough so that ears per plant average 0.3 to 0.7 across the entire experiment.
- Measurement: count the number of ears with at least one fully developed grain and divide by the number of harvested plants.

5.3.3 Anthesis-silking interval (ASI)

- Heritability: medium, maintaining a reasonably high level under severe flowering stress.
- Relationship with grain yield: high under flowering stress.
- Selection is for a reduced or even negative ASI.
- Stress type: to be measured under flowering drought stress; heritability and genetic variance is the largest when flowering stress is intense enough so that ASI averages 4 to 5 days across the entire experiment.
- Measurement: determine the number of days from sowing until 50% of the plants have extruded anthers (anthesis date, AD), and the number of days from sowing until 50% of the plants show silks (silking date, SD); calculate: ASI = SD - AD.

5.3.4 Leaf senescence

- Heritability: medium.
- Relationship with grain yield: medium under grain-filling stress.
- Selection: for delayed leaf senescence (stay-green).
- Stress type: grain filling stress.
- Measurement: score on a scale from 0 to 10, dividing the percentage of estimated total leaf area that is dead by 10.

1 = 10% dead leaf area	6 = 60% dead leaf area
2 = 20% dead leaf area	7 = 70% dead leaf area
3 = 30% dead leaf area	8 = 80% dead leaf area
4 = 40% dead leaf area	9 = 90% dead leaf area
5 = 50% dead leaf area	10 = 100% dead leaf area

Remarks: Leaf senescence should be scored on 2-3 occasions 7-10 days apart during the latter part of grain filling.

5.3.5 Tassel size

- Heritability: medium to high.
- Relationship with grain yield: medium under flowering stress.
- Selection: for a smaller tassel with fewer branches.
- Stress type: this is the only trait that can be measured under well-watered conditions but is indicative of drought tolerance at flowering stage.
- Measurement: score on a scale from 1 (few branches, small tassel) to 5 (many branches, large tassel).

Remarks: advisable only with lines that have an inbreeding degree of at least S_1; more difficult to determine with full vigor material. Two independent scores are recommended.

5.3.6 Leaf rolling

- Heritability: medium to high.
- Relationship with grain yield: medium to low.
- Selection: for unrolled leaves.
- Stress type: flowering stress.
- Measurement: score plots on a scale from 1 to 5.

1 = unrolled, turgid	4 = rolled leaf rim covers part of leaf blade
2 = leaf rim starts to roll	5 = leaf is rolled like an onion
3 = leaf has a the shape of a V	

Remarks: to be measured before flowering when leaves are still more upright; leaves are less likely to roll after flowering when they become more lax and thicker. Two to three scores are recommended.

5.3.7 Additional remarks

- The more high quality information available, the better the likelihood of determining the drought tolerance of a genotype.
- Even if only grain yield is measured, anthesis date needs to be known so that drought-escaping genotypes can be distinguished from drought-tolerant ones.
- With consistent selection for short ASI under flowering stress, the frequency of male-sterile genotypes may increase, because delayed anther extrusion may be confused with a short ASI. This is especially important when evaluating inbred lines under drought.
- Many other secondary traits for drought tolerance were evaluated by CIMMYT but proved to be of low heritability, among them: leaf and stem elongation rate, canopy temperature, leaf photo-oxidation, leaf chlorophyll concentration, predawn leaf water potential, and seedling survival under drought.
- Other traits evaluated by CIMMYT were heritable, but proved to have no relationship with grain yield under drought: osmotic adjustment, leaf erectness.

5.4 Secondary traits that help to identify low N tolerance

The following traits are recommended for use in a low N breeding program. They are listed in order of decreasing priority.

5.4.1 Grain yield

- Heritability: medium.
- Relationship with grain yield: high.
- Selection: for high grain yield.
- Stress type: N stress that results in less than 50% of the yield normally obtained under well-fertilized conditions.
- Measurement: shelled, adjusted for grain moisture.

Remarks: Shelling percentage varies considerably under low N. Grain weight, not ear weight, should be used for calculating grain yield.

5.4.2 Ears per plant

- Heritability: high
- Relationship with grain yield: high under severe N stress.
- Selection: for more ears per plant (reduced barrenness).
- Stress type: to be measured under severe N stress (preferably at a yield level 25 to 35% of well-fertilized yield); heritability and genetic variance increase as the severity of the N stress increases.
- Measurement: count the number of ears with at least one fully developed grain and divide by the number of harvested plants.

5.4.3 Leaf senescence

- Heritability: high.
- Relationship with grain yield: medium to high.
- Selection: for delayed leaf senescence (stay-green).
- Stress type: N stress that results in less than 50% of the yield normally obtained under well-fertilized conditions.
- Measurement: score on a scale from 0 to 10, dividing the percentage of the estimated total leaf area that is dead by 10.

1 = 10% dead leaf area	6 = 60% dead leaf area
2 = 20% dead leaf area	7 = 70% dead leaf area
3 = 30% dead leaf area	8 = 80% dead leaf area
4 = 40% dead leaf area	9 = 90% dead leaf area
5 = 50% dead leaf area	10 = 100% dead leaf area

Remarks: Leaf senescence should be scored on 2-3 occasions 7-10 days apart during the latter part of grain filling.

5.4.4 Anthesis-silking interval (ASI)

- Heritability: medium.
- Relationship with grain yield: medium under severe N stress.
- Selection: for reduced or negative ASI.
- Stress type: to be measured under severe N stress (25 to 35% of well-fertilized yield); heritability and genetic variance increase as N stress becomes more severe.
- Measurement: determine number of days from sowing until 50% of the plants have extruded anthers (anthesis date, AD), number of days from sowing until 50% of the plants show silks (silking date, SD); calculate: ASI = SD - AD.

5.4.5 Additional remarks

- Anthesis date should be known during selection, because maturity in some instances is correlated with grain yield under low N.
- The yield component that is reduced the most under low N stress is grain number per ear. It is probably not worth measuring separately, because most of the information it conveys is included in grain yield itself.
- Drought and low N tolerance are related inasmuch as selection for drought tolerance improves performance under low N stress as well. Drought tolerant varieties have fewer barren plants and delayed leaf senescence under low N stress (Lafitte and Bänziger 1997). Thus, the overlap in the list of secondary traits recommended for each stress is not surprising.
- Several secondary traits for low N tolerance were evaluated by CIMMYT but proved to be of low heritability, among them: leaf chlorophyll concentration.
- Other traits evaluated by CIMMYT were heritable but proved to have no relationship with grain yield under low N: leaf area of the ear leaf.
- Other traits were evaluated by CIMMYT, were heritable and were related with grain yield under low N, but had negative consequences: plant height (increased lodging).

5.5 Selection indices - Combining information on secondary traits with grain yield

There are several good reviews on the theory and use of selection indices (Baker 1986; Lin 1978). A selection index summarizes the worth of genotype by making use of information from different traits such as grain yield, ASI, senescence, etc. To add together traits measured in different units, the phenotypic values, P_i, are usually standardized, as:

$$P_i = (x_{ij} - m_i)/s_i \qquad [5]$$

where m_i and s_i are the mean and standard deviation of trait i in a population, and x_{ij} is the value of the trait i measured on genotype j.

A selection index I in its simplest form can then be written as:

$$I = b_1P_1 + b_2P_2 + \ldots b_nP_n$$

where P_i is the observed standardized value of the trait i and b_i is the weight given to that trait in the selection index. Weights may be chosen based on the relative economic value of each trait or based on the relative value of each trait as an indicator of drought or low N tolerance. The genotype with the largest value for I is the best genotype. When defining weights, one has to be careful that they have the right sign: positive where larger values are desired (e.g. grain yield), negative where lower values are desired (e.g. lodging, ASI).

CIMMYT has developed software that calculates such a selection index (ALPHA) using MSTAT data files. AGROBASE® is another program that can calculate selection indices. These programs standardize the data for the traits included in selection. The breeder assigns weights to these traits, based on their variance, their correlation with other traits, the significance of the genotype term in their ANOVA (or their hertability), and their relative importance in contributing to drought or low N tolerance. Traits that are genetically variable and that are known to be relatively more important for assessing drought or low N tolerance are weighted more strongly. The program then calculates the index as a single measure of the drought or low N tolerance of that genotype.

Typical weights allocated in a drought breeding program are:

Grain yield	weight = 5	sign = + (increased grain yields)
Ears per plant	3	+ (increased no. of ears per plants)
ASI	2	- (decreased ASI)
Leaf senescence	2	- (decreased leaf senescence)
Tassel size	2	- (decreased tassel size)
Leaf rolling	1	- (decreased leaf rolling)

Typical weights allocated in a low N breeding program are:

Grain yield	weight = 5	sign = + (increased grain yields)
Ears per plant	2	+ (increased no. of ears per plants)
Leaf senescence	2	- (decreased leaf senescence)
ASI	1	- (decreased ASI)

Anthesis date and plant height are often included in the selection index so that the selected fraction of the population does not become either later, earlier, or taller than the original, unselected population. In drought experiments, there is a risk that earlier (drought escaping) genotypes will be selected. Comparing the mean of the selected fraction with the mean of all genotypes being evaluated can help to prevent undesirable changes in the germplasm.

With the selection index introduced above, weights are chosen by the breeder. Optimal weights for each trait used in selection can actually be calculated, based on the phenotypic and genotypic covariance between the trait and grain yield. Predicted progress for grain yield can thereby be maximized (Lin 1978). The problem of this mathematically correct approach is that there is no software available that routinely does the calculations, which become especially complex with improved experimental designs.

5.6 Combining information from various experiments

For selection purposes, results from managed drought or low N experiments need to be combined with experiments conducted under well-fertilized, well-watered conditions to select for genotypes that do well over a range of environments.

We recommend the following **procedures to weight drought and low N experiments in a way the breeder can control and which truly fit the importance of these stresses in the target environment**:

1. Combine data **within** a drought or low N experiment using a selection index (for example, ALPHA). Depending on the type of experiment, the selection index gives you **one value for drought tolerance** or **one value for low N tolerance**.

2. **Summarize data from the other non-stressed sites** so you have, for example, one value for grain yield under non-stressed conditions, one value for lodging, one value for *Exserohilum turcicum* resistance, etc.

3. **Select based on the relative priority of established breeding goals using a selection index across all characteristics.**

A more simple but less effective method is to take **the average of the rankings of genotypes in each environment**. This relatively crude method will effectively weight each environment equally. Increased weighting on a single environment can be accomplished by multiplying the rankings from that environment before averaging.

6 Breeding strategies

6.1 Introduction

Developing maize genotypes with tolerance to drought and N stress is complex. This is due to various factors, including the largely polygenic nature of the tolerance, the typically low frequency of tolerance alleles in most maize germplasm, and the difficulties commonly encountered in field evaluations. Important considerations in establishing a selection program for stress tolerance should be whether OPVs, hybrids or both types of products are needed, and what human, financial, and physical resources are available for experimental work. Additional important factors include the choice of germplasm, breeding methodology, selection environments, and essential data to collect.

6.2 Choice of germplasm

The selection of appropriate germplasm is critical, requiring careful consideration of all available information. A wrong choice cannot be corrected by using sound and efficient breeding methodologies. There are several approaches a breeder can take to develop drought and low N tolerant germplasm.

6.2.1 Improving locally adapted, elite germplasm for drought and low N tolerance

CIMMYT evaluated a wide range of landraces for drought and low N tolerance, but there were few (about 3%) that compared favorably with elite, adapted germplasm for drought or low N tolerance, and even fewer that compared favorably with elite, adapted germplasm under high yielding conditions. Additionally, we have found genetic variability for drought and low N tolerance in all types of elite germplasm. Thus, **improving adapted, elite germplasm for drought and low N tolerance** is probably nearly always better than working with landraces. A compromise approach would be to create synthetic populations from local landraces and improved, adapted varieties.

6.2.2 Improving non-adapted but drought and/or low N tolerant populations for local adaptation

CIMMYT-Mexico and much more recently CIMMYT-Zimbabwe and CIMMYT-Kenya have developed germplasm with high levels of drought and/or low N tolerance. A breeder may want to use such germplasm or any other known source of drought or low N tolerance. Such materials may not be well adapted in terms of disease resistance, maturity, etc., to the target environment. Screening for general adaptation by the breeder may be necessary, and only the best adapted among the introduced stress-tolerant materials should be used. Adaptation can usually be further improved through selection, and for many breeders it is easier to select for adaptation (suitable maturity, disease resistance, yield potential) than for drought or low N tolerance.

6.2.3 Formation of new breeding germplasm through introgression

The third and more complex strategy is to develop a new breeding population by introgressing locally adapted germplasm with source drought or low N tolerant germplasm. This approach raises several issues: which source germplasm should be used? What proportion of local and source material is appropriate? How much recombination is necessary before the population is ready for intense selection? And how can molecular markers facilitate this process?

6.2.3.1 Which source population(s) to use?

A breeder should check first the information that is already available for the potential source germplasm:

- General adaptation: lowland tropical, subtropical/midaltitude, highland, temperate.
- Grain color and grain texture.
- Maturity: it is better to use heat units than calendar days for characterizing the maturity of a genotype.
- Disease resistance.
- Tolerance to abiotic stresses.
- Heterotic pattern and response.
- Combining ability: it takes a lot of effort to identify a line with good combining ability, and if possible only lines with proven combining ability should be introduced.
- Other value-added traits.

A breeder should introduce only germplasm that matches farmers' preferences (grain color, texture, size) and the environment (disease resistance, maturity, need for acid soil tolerance etc.). If hybrids are the desired product, the heterotic response of an introduced line should be known so that the line can be used in the appropriate heterotic group.

6.2.3.2 Evaluation of source population(s) in the target environment

Once source germplasm is introduced, it should be evaluated in the target environment. Such an evaluation might be of:

- Population or line *per se*: This should be done not only for gathering initial data on maturity, performance, disease resistance, etc., but also for increasing seed of the most promising germplasm.
- Selfs of a population: to select directly the most highly adapted fraction of a population.
- Population x local tester topcross combinations.
- Line x local tester topcross combinations.
- Diallels of local and exotic populations or lines.

The frequency of useful 'exotic' lines is typically low, and a breeder should invest only in germplasm that proves to have as many valuable traits as possible. Even if only germplasm that meets certain basic requirements such as maturity, grain color and texture, and disease resistance (see previous paragraph) is introduced, a breeder should likely invest only in about 10-25% of all germplasm introduced.

6.2.3.3 What proportion of 'local' versus 'source' germplasm?

The proportion of 'source' germplasm will depend on the balance between the adaptation of the source germplasm and the drought or low N tolerance level of the local population(s). If the local and source populations do not differ much in performance, the F_2 population should be used as a base foundation population. If the difference in performance between parents is large, one to three backcrosses to the superior germplasm are appropriate.

6.2.3.4 How much recombination in 'local x source' populations?

It is beneficial to recombine 'local x source' populations for 2-3 generations with mild phenotypic selection for general adaptation following the introgression process, before intense inbreeding and selection are initiated (Geadelmann 1984). This breaks up linkages between desirable and undesirable genes. During recombination it is important to maintain an adequate effective population size (usually > 200 successful pollinations) to avoid genetic drift.

6.3 Breeding schemes

6.3.1 Integrated strategies for developing stress-tolerant maize germplasm

The extent to which selection for stress tolerance can be included in a breeding program depends on the breeding scheme used. A few strategies will be outlined below, though there are many others that can be employed. **In any breeding program, however, the following need to be clearly defined:**

- The type of product to produced: OPV, hybrid, topcross hybrid, etc.
- The most important characteristics of the product: maturity, grain characteristics, necessary stress tolerance and disease resistance, etc.
- The strategy for developing and deploying the product.

All breeding programs use a step-wise selection procedure to identify the best performing progenies, given limited resources. First, a large number of progenies are evaluated with few replicates and at few sites **(screening)**, then the more successful progenies, or their descendants, are evaluated with more replicates and at more sites **(testing)**.

Screening: If drought and/or low N tolerance are important breeding goals, evaluation under these stresses should be included in the screening phase, and the results should be combined with results obtained under unstressed conditions. Only the best germplasm (i.e., genotypes that possess stress tolerance and have good yields under optimum conditions) should be advanced to the testing phase.

Testing: The testing phase should include sites that are representative of conditions under which farmers grow maize. If drought and N stress are frequent in farmers' fields, these conditions must be included. We suggest you include results from both managed stress sites and randomly stressed sites (e.g., farmers' fields) during the testing phase. These multilocation trials need to be set up in a manner that considers the difficulties of stressed sites. One should not hesitate to increase the number of replicates and reduce the number of entries in such trials.

6.3.1.1 Example 1: A variety evaluation program of a national program that does not have its own maize breeding nursery

A certain national program may routinely evaluate about 60 genotypes introduced from other breeding programs for suitability to its country's growing conditions. Drought and N stress are important constraints for farmers in this country.

Conventional approach: Conventionally, this program would screen the 60 genotypes at 2 to 3 experiment stations under well-fertilized and perhaps irrigated conditions (screening phase). The best 12-16 genotypes would be increased and evaluated at 6 to 8 well-fertilized, rainfed sites during the following 2-3 years, using 4 row plots and 3 replicates (testing phase). The breeder observes that results from drought-stressed sites during the testing phase are often not significant for entry effect and that considerable GxE interactions occur because of random drought stress. The release decision is therefore based mainly on performance under high yielding, well-watered conditions.

An approach that considers the demand for drought and low N tolerant germplasm: Under this approach, this program would screen the 60 genotypes under well-watered, well-fertilized conditions (main season), under managed N stressed (main season) and under managed drought stressed conditions (dry season), for a total of three managed environments. Disease ratings are taken in the main season experiments. The best 20 genotypes from the first year would be reevaluated in the second year under the same three managed environments. The best 4-6 genotypes that have a high yield potential, are drought and low N stress tolerant, and have the desired disease resistance and grain texture, based on two years' results, are increased for multilocation trials. Note: under this approach the breeder can apply a more severe selection pressure after the screening phase, because stresses that are relevant in the target environment were included in the screening phase. The 4-6 genotypes are then evaluated at 6-8 locations, using 6 replicates per site, 2-row plots, and a fertilized and unfertilized treatment at each site. Because of the higher number of replicates during multilocation testing and the fewer and better pre-screened entries, this approach uses about the same resources as the conventional approach, but will likely result in cultivars that are better adapted to farmers' conditions.

6.3.1.2 Example 2: A hybrid breeding program

A certain national program may routinely produce its own test crosses, evaluate them, advance the best lines, and produce hybrids that are then evaluated at several locations. Again, drought and N stress are important constraints for farmers in this country.

Conventional approach: Conventionally, this program may annually develop about 200 S_3 lines with good disease resistance and per se performance. They are crossed to 2 to 3 testers and the test crosses evaluated at 3 to 5 experiment stations under well-fertilized and (possibly) even irrigated conditions (1st screening phase). Progenies of the 10 best lines (good combining ability under high-yielding conditions, good disease resistance, *per-se* performance, standability, and grain characteristics) are chosen for making single- and triple-cross hybrids. These hybrids are then evaluated at 3 to 5 experiment stations under well-fertilized and perhaps irrigated conditions (2nd screening phase). The best 12-25 hybrids enter multilocation testing at well-fertilized, rainfed sites during the following 2-3 years, using 4 row plots and 3 replicates (testing phase). The breeder observes that results from drought-stressed sites during the testing phase are often not significant for entry effect and that considerable GxE interactions occur because of random drought stress. The release decision is therefore mainly based on performance under high yielding, well-watered conditions.

Approach that considers the demand for drought and low N tolerant germplasm: Under this approach, this breeding program includes performance under managed drought stress when developing the S_3 lines. For instance, 1,000 S_1s could be screened in an unreplicated trial under managed drought (using an augmented design) and only the best 200 advanced to S_2, using remnant seed of the same S_1s. Disease resistance and per se performance are considered while developing S_3s from these S_1s. The S_3s are crossed to 2 to 3 testers and the test crosses evaluated at two sites under well-fertilized, well-watered conditions, under managed flowering drought stress, and under managed N stress (1st screening phase). Progenies of the best lines (good combining ability under high-yielding, drought stressed and N stressed conditions, good disease resistance, *per se* performance, standability and desirable grain characteristics) are chosen for making single- and triple-cross hybrids. These hybrids are evaluated at two sites under well-fertilized, well-watered conditions, under managed flowering drought stress, under managed grain-filling drought stress, and at two sites under managed N stress (2nd screening phase). The best 4-6 hybrids enter multilocation testing in both fertilized and unfertilized fields on-farm using 6 replicates per site. Again, because of the higher number of replicates during multilocation testing, the fewer and better pre-screened entries, this approach uses about the same resources as the first approach, but will likely result in hybrids that are better adapted to farmers' conditions.

6.3.2 Population improvement schemes

Maize provides a wide array of options with respect to breeding methodologies. One choice is between intrapopulation and interpopulation improvement methods. Within intrapopulation improvement methods, alternatives are:

- Individual plant versus family selection.
- Non-inbred families versus selfed progenies.
- *Per se* performance versus test cross performance.

Within interpopulation improvement methods, alternatives are:

- Test crosses involving individuals versus families.
- Half-sib versus full-sib test cross progenies.
- Parental versus non-parental testers.

6.3.2.1 Individual plant selection schemes

Two common procedures are simple mass selection and stratified mass selection (Gardner 1961). The procedures are not recommended for traits with relatively low heritability, such as grain yield under drought or low N stress. They can be quite effective for highly heritable traits, such as selecting for disease resistance after introgressing an exotic stress-tolerant but disease susceptible genotype into well-adapted, disease resistant germplasm. One selection cycle can be completed every season. The experimental area can be stratified to reduce differential environmental effects. Tassels of undesirable plants can be eliminated before flowering to prevent pollen contaminating selected plants. A few cycles of mass selection may successfully eliminate the most susceptible fraction of the population before switching over to a family-based improvement method. This is a good option where human, financial, and physical resources are limited.

6.3.2.2 Family-based selection: *per se*

Family-based selection methods result in greater gains when traits under selection are complex and of low heritability, but are more demanding in resources, record keeping and overall management. Progenies such as half-sib, full-sib, S_1, S_2, etc., are evaluated. Progress can be expected from any one of these methods. The choice of method will be guided by the availability of off-season test sites, the ability to store remnant seed, the choice of product (variety, hybrids or both), desired traits, heritability, progeny seed quantities, the degree of control over pollination (both parents or only one parent), and the time required to complete a selection cycle.

Half-sib improvement methods in which detasseled half-sibs (females) are pollinated with pollen from a bulk of all half-sibs (male) are commonly practiced using an unreplicated layout. Because the females are detasseled, ASI cannot be observed. For drought and low N tolerance improvement, it is therefore more desirable to plant replicated trials of half-sib progenies and use remnant seed for recombination of selected families. Heritability of yield from half-sib progenies is lower than that for other types of progenies. However, where resources are limited, this may be the most cost-effective selection scheme.

Full-sib family recurrent selection has been used extensively at CIMMYT to improve populations for drought and low N tolerance. Replicated trial sets of full-sib progenies are evaluated under drought, low N and well-watered, well-fertilized conditions. Selection is made based on performance in all environments and considering other factors such as disease resistance, grain texture etc. A single cycle of selection requires at least two seasons to complete.

Selfed progenies: When breeding procedures are based on selfed progenies, it takes longer to complete a cycle of selection, but this approach significantly improves tolerance to inbreeding over time. Formation of many S_1 or S_2 progenies is recommended. These can be prescreened in unreplicated observation nurseries under drought or low N and the selected fraction (perhaps only 30% of the original progenies) can be examined in more detail in replicated evaluations under, say, drought-stressed, N stressed and well-watered, well-fertilized conditions. Where prescreening in the main season is possible, disease susceptible progenies can be eliminated. Seed supplies may become limiting. This can be solved by using selected S_2 ear bulk seed developed from each S_1 progeny. To maintain population gains over longer periods, it is recommended that no fewer than 20-40 inbred progenies be recombined.

6.3.2.3 Family based selection: test crosses

Here the test crosses of S_2 or more inbred progenies are evaluated. The time required to complete a cycle of selection will thus depend on the materials that are test-crossed. Such schemes are useful when the emphasis is on combining ability, hybrid-oriented germplasm, and the integration of population and hybrid development. They can also be recommended where the need is to identify superior, early generation lines for further inbreeding or improving a population *per se*. Evaluation for stress tolerance can be emphasized during the formation and prescreening of selfed progenies as well as during test cross evaluation.

6.3.2.4 Interpopulation improvement alternatives

Two commonly used methods discussed here include reciprocal recurrent selection/half-sibs (RRS-HS) (Comstock et al. 1949) and reciprocal recurrent selection/full-sibs (RRS-FS) (Hallauer and Eberhart 1970; Hallauer 1973). Such schemes result in improved populations and superior OPV products as well as improving hybrid-oriented features of the two populations by increasing the level of heterosis between them. In addition these schemes allow the extraction of early-generation lines with good general combining ability (GCA), provide a sound basis for recycling early generation lines, identify superior testers on a continuous basis, and may identify new

conventional and non-conventional hybrids. The schemes are not particularly suitable if the populations do not tolerate inbreeding, and the *per se* performance of lines and parent populations is ignored during selection. The original schemes also recommend evaluating S_0 test crosses and recombining the parental S_1 seeds of good performing plants. The modified schemes attempt test crosses (HS or FS) on S_1 or S_2 progenies and also permit selfed progeny evaluation for elimination of undesirable progenies. The RRS-FS schemes have an added advantage over RRS-HS, in that only 50**%** of resources are spent on test cross progeny evaluation trials. Both original and modified schemes permit selection for drought and low N at one or more stages during the selfed progeny regeneration and evaluation stages and during the evaluation of test cross progenies. These types of interpopulation improvement schemes are not a necessary requirement for hybrid development, but from a long-term perspective they should generate useful early generation lines.

6.3.3 Development of drought and low N tolerant lines and hybrids

Types of hybrids emphasized (topcross hybrids, double-, triple-, or single-cross hybrids) will depend on the stage of hybrid development and seed industry infrastructure, but an evolution from non-conventional to conventional and from multiparent to two-parent hybrids seems logical. Populations improved for drought or low N tolerance are useful sources for extracting drought or low N tolerant inbred lines.

6.3.3.1 Line - hybrid correlations

The relationship between the performance of inbred lines and their hybrids is an important issue in hybrid development. Inbred line information indicative of hybrid performance is desirable to reduce hybrid trial evaluations. Lafitte and Edmeades (1995) reported that the correlation between S_2 *per se* and topcross performance under low N was only 0.22. Betran et al. (1997) have reported correlations of around 0.4 between S_3 *per se* and topcross performance for some stress-related traits under drought, indicating that inbred lines insufficiently predict hybrid performance under drought or low N. The practical implication of these findings is that drought or low N evaluations of lines may be justified in early generations when numbers of progenies are yet very large, but the performance of advanced lines is best evaluated in hybrid combination.

6.3.3.2 Choice of appropriate testers

The choice of testers is a critical yet difficult decision in hybrid development. Appropriate choices will have a strong effect on the outcome of a program designed to identify stress-tolerant hybrids. Testers can be inbred, non-inbred, populations, synthetics, or hybrids. The choice involves a blend of theoretical and practical considerations. For example, should one use a broad or narrow genetic base tester, high or low yielding, one with high or low frequency for stress tolerance traits, good or poor GCA, one or several testers, and related or unrelated testers? Testers with a low gene frequency for the selection traits emphasized are theoretically attractive but are not commonly used, particularly regarding yield. For drought and low N selections, they might be more practical, since many conventionally developed testers have never been selected for drought or low N tolerance. **A desirable tester must facilitate discrimination among genotypes for combining ability and desirable traits, simultaneously identify useful hybrid products for direct use, and be compatible with a practical maize breeding program** (Vasal et al. 1997). For practical purposes, we recommend using the same testers for evaluating combining ability under drought or low N stressed conditions, as they are used for evaluating combining ability under well-watered, well-fertilized conditions.

6.3.3.3 Dosage effects

Preliminary results on the genetic control and modes of action for drought and low N tolerance show the following:

- Lines are more affected by drought and N stress than hybrids.

- As drought stress increases, so does the importance of general combining ability and additive genetic effects.

- In contrast to drought, non-additive effects are more important under low N stress.

- Dosage effects are important under drought but not under low N stress, suggesting the need for including drought tolerant parents on both sides of the hybrid to achieve acceptable drought tolerance, where stress is severe.

- Line-hybrid correlations are generally lower under stress than non-stressed conditions.

6.3.3.4 Line and hybrid improvement by introgression

Here we discuss strategies to improve line and hybrid performance. Our most important decision is to identify the source germplasm (lines, synthetics, populations, hybrids, etc.) most likely to contribute the most favorable genetic factors for drought or low N tolerance to the elite recipient line or hybrid. Several methods of selection of the donor source have been described by Beck et al. (1997). The objective is to identify source germplasm with the highest frequency of favorable dominant alleles that are not present in an elite hybrid. A detailed discussion of these methods is complex and we refer readers to these sources for further information.

A pragmatic approach that is taken by many breeders is first to evaluate source inbred lines for *per se* adaptation to the target environment. Again, many lines, maybe 60 to 80%, may be discarded in this step, and only lines with desirable disease resistance, maturity, and grain characteristics are then crossed to the local tester lines and their combining ability and heterotic response determined under managed stress and unstressed conditions.

Introduced stress tolerant lines may be used directly as one of the parents in a hybrid that is then released. More often, however, stress-tolerant lines need to be introgressed into local germplasm. After the initial cross between source and recipient line, selection and or inbreeding can be initiated either immediately or after one or more recombinations or backcrosses. Repeated recombination before initiating inbreeding increases the chances of obtaining inbreds with stress tolerance *and* good agronomic performance. Backcrossing is advantageous if one parent has more loci with favorable alleles than the other, if the parents are diverse, or if the level of dominance is high (Dudley 1984).

6.4 Biotechnology: Potential and constraints for improving drought and low N tolerance

6.4.1 Biotechnology applications in maize breeding programs

Biotechnology tools continue to develop rapidly, opening new possibilities. So far, in most maize breeding programs, applications will be for:

- **Fingerprinting of inbred lines**: the information can be used to identify lines used as parents in a hybrid, or to predict heterosis in crosses by estimating genetic distance between parents.

- **Line conversion**: a trait (or traits) of interest is transferred from a donor line to a recipient elite inbred line. Where a single trait is to be transferred, marker-assisted backcrossing can reduce the need for backcrosses from the usual four to five to around two. At the same time, the amount of "linkage drag" associated with transfer of unwanted parts of the donor genome to the recipient line is reduced (Ribaut and Hoisington 1998).

6.4.2 Marker-assisted selection (MAS) for drought and low N tolerance

Marker-assisted selection will be an effective way to save time in breeding if:

- The heritability of the trait is high and field evaluation is very costly or simply cannot be done at your location.

- Environmental effects are significant, heritability is low, and classical selection is expensive or slow, or if the conditions for selection are only present occasionally (e.g., selection for drought tolerance in the rainy season).

- If you want to backcross a known gene into an inbred line as rapidly as possible.

Because of the importance of anthesis-silking interval, CIMMYT tried to identify quantitative trait loci (QTL) for ASI and yield components under drought in maize. Six QTL were identified on chromosomes 1, 2, 5, 6, 8 and 10, accounting together for approximately 50% of the phenotypic variability of ASI. The QTL segments were stable over years and stress levels. In contrast, all but two yield QTL were inconsistent in their position in the genome in different water regimes. At one important genomic position, the allele contributing to a reduction in ASI also contributed to a grain yield decrease, and for this reason CIMMYT's marker-assisted selection strategy for drought tolerance is now based on an index of best QTL for both traits (Ribaut et al. 1996; 1997a; 1997b; 1997c).

Preliminary results suggest that MAS based on a strategy combining both ASI and grain yield QTL identified under drought could be a powerful tool to improve drought tolerance in tropical maize inbred lines and perhaps also in open-pollinated populations. It is noteworthy that, when mapped in the same F_2 population as was ASI under drought, ASI under low N has several QTL in common with those observed under drought. Thus, we can expect that improvements in ASI using marker-assisted selection should also result in improvements in tolerance to low N.

The advent of marker assisted selection opens up real prospects for new strategies in breeding that combine conventional and marker technologies that suit the genetics of the both the trait and the plant.

- Molecular markers allow the handling of very large numbers of genotypes during backcrossing while giving the breeder the tools to quickly reduce those numbers, based on their genomic composition.

- Large-scale F_2 marker assisted selection schemes for developing elite, trait-enriched populations fixed for traits of interest and segregating elsewhere are an exciting prospect for programs dedicated to developing broadly-based, elite, value-added germplasm.

- Marker-assisted selection opens the possibility of testing the desirability of specific traits by developing near isogenic lines that differ only for the DNA segments associated with the trait of interest—a tremendous tool for physiologists involved in testing the value and importance of secondary traits in selection.

7 The role of the farmer in selection

It goes without saying that if the variety being developed for improved tolerance to drought and low N is unacceptable to farmers for other reasons and is not adopted, all the research work invested in that variety will be wasted. It is critically important, therefore, that farmers be involved in the selection and testing process, and that researchers pay careful attention to farmers' views on what constitutes an appropriate and attractive maize variety under their circumstances.

7.1 What is farmer participatory research and why is it important?

Farmer participatory research represents:

- A dialogue between farmers and scientists to solve agricultural problems.
- A way to increase the impact of agricultural research by developing technologies that are more widely adopted.
- A path to more productive, stable, equitable, and sustainable agricultural systems.

The goal of this chapter is to present basic concepts and methods for incorporating a farmer participatory approach into breeding for drought and low N tolerance in maize.

7.2 What is new about farmer participatory research?

Farmer participatory research emphasizes three aspects: farmers' knowledge, farmer experiments, and farmer exchange of information and technologies.

7.2.1 Farmers' local knowledge

Farmers have an extensive and well-developed knowledge base on their environments, crops, and cropping patterns built up over many seasons and even generations. Farmers' local knowledge is often made up of **perceptions**, or mental images based on repeated observations in the normal course of life and work on the farm. This information is normally organized into hierarchical categories (also called "taxonomies") with names and defined properties. Farmers normally have taxonomies for such areas of direct interest as crops, crop varieties, soils, insects, among others. Farmers' knowledge also comprises many **rules of thumb**—logical propositions that relate two events in a cause and effect manner. This applies mainly to things that can be easily observed and are relatively straightforward. Drought is one such phenomenon, whereas a steady decline in soil fertility over the years may pass almost unnoticed. Where things are hard to see with the naked eye or have multiple causal factors, farmers often have incorrect knowledge or none at all. Farmers' knowledge is dynamic, changing in response to new observations and evolving circumstances.

7.2.2 Farmer experiments

Farmers carry out experiments on their own and generate innovations – not every farmer does the same things on the land, so what are the reasons for the evolution and diversity of practices? Farmers' experiments or comparisons of alternatives play a key role in their livelihoods, since they can thus evaluate new and unproved technologies without jeopardizing scarce resources. Farmers' and scientists' experiments often differ in that farmers' experiments usually lack a control treatment, include many factors that may be modified simultaneously, and are usually not replicated in the formal sense. The main source of data is the farmers' own observations, and they lack the tools to observe many things scientists traditionally observe, as well as the statistical methods to test probabilities. Nonetheless, they have a well-developed idea of risk, and hence an informal understanding of probabilities.

7.2.3 Farmer exchange of information and technologies

Farmers actively exchange information and technologies among themselves. This is usually an informal, even social process, but it is perhaps the major way in which a farmer learns new ideas, perceives long-term risk, obtains new seed, etc. Farmers' exchange of information can also happen through mechanisms such as migration, off-farm work and casual contacts. Social barriers sometimes constrain these exchanges in unexpected ways, such as barriers between social castes, fear of witchcraft, or a fear of generating envy.

7.3 Participatory methodologies

7.3.1 With whom should we work?

How do we select participants? The answers we get and how representative they are will depend very much on how we select our informants. The following are possible strategies for selecting farmer partners:

- **Incidental selection**: Persons whom we encounter and are willing to talk to us, without any effort on our part to identify them.
- **Selecting key contacts**: We select participants based on well-defined criteria and with the help of local contacts who know the scene.
- **Random-selection**: We choose participants using some sampling procedure.
- **Self-selection**: Persons volunteer to participate. For example, when there is an open invitation to an event they come, or they make contact with the scientist on their own.

7.3.2 How to interact with participants

Types of interviews/interactions include **individual** and **group**. The first involves a one-to-one interaction between the scientist and the farmer, when often a large number of questions get asked of the farmer. In group interactions, the scientist meets with several participants. Here the objective is to ask relatively few questions, generate discussion among farmers, and identify areas of agreement and disagreement.

7.3.3 Methods for grouping farmers and responses

7.3.3.1 Grouping farmers

Gender and wealth are fundamental in classifying responses on varietal preferences. Wealthy farmers may handle issues such as risk differently from those who are poor, and they may have special varietal preferences. This makes it necessary to classify a known group of farmers into wealth categories. Informants should know the community well, should include both males and females, and should help develop a list with the names of the farmers to be ranked. Informants should be asked to list the characteristics of prosperous and poor farmers, as well as those of intermediate wealth. Then the name of each farmer should be read to the informants, who should assign him/her to one of the three categories of wealth. Often the use of cards depicting the characteristics of each wealth rank can be used to speed the process.

7.3.3.2 Grouping responses

Two approaches are frequently used to determine characteristics of local varieties and assess their importance to farmers. One involves groups and the other individuals. The methods are the same for both:

- Identify all local varieties in the community of interest.
- Identify positive and negative traits that farmers consider important.
- Generate a comprehensive list of the traits.
- Rate the traits in terms of their importance to farmers.
- Rate the performance of each variety with respect to each trait.

Since the approaches are basically the same, we will focus on the group approach. For this approach several groups should be formed, each with individuals that share socioeconomic or gender traits considered important. For example, groups may comprise one of males and one of females, a group of farmers with small land holdings and one of farmers with large land holdings, etc. The idea is to try to ensure that communal diversity is fairly reflected. In addition, dividing farmers into homogeneous, contrasting groups allows assessment of the variation in farmer-perceived traits for varieties and a sense of the importance each group assigns to specific traits.

Each group is asked to compose a list of maize types or varieties sown. Effort should then be made to identify types or varieties that are the same but go by different names. (For each pair of varieties that can be formed from the list, ask whether they are the same or different. For example, for a set of three varieties, A, B, and C, we would ask: is variety A the same as variety B, is variety A the same as variety C, and is variety B the same as variety C?) Once "synonyms" have been eliminated, ask the group to list the positive and negative traits of each variety. One method to accomplish this is, for each variety, to request a show of hands on who has discontinued its use and why. With the farmers' answers, compile a list of traits they consider important. An example of the type of data that results from this exercise is presented in Tables 1 and 2. Compiling a comprehensive list from the farmers' answers may present a few problems, since farmers may refer to the same characteristics in different ways; once again, judgement is needed to identify synonyms.

To assess the importance of these traits and farmers' general demand for certain traits, supplement the list generated above with a few traits considered important by researchers but not mentioned by farmers (for example, traits related to response to N or to drought). Then, ask the group to rate each trait through a show of hands of those who consider the trait very important, then of moderate importance, and finally, those who think it is not important at all. (Alternately, voting can be simplified by asking who considers the trait important or not.)

Different groups within the same community may have different traits in their lists. This can be interpreted as indicating that they weight traits differently. Ideally, one would like to have the same list for all groups, and then proceed with the rating exercise again by group, to obtain systematic comparisons across groups. However, this will require added time, breaking the flow of the exercise, and dividing it into two sessions. In addition, if the list is too long, higher priority may have to be given to the traits mentioned in all groups, and lower importance to those mentioned only in one or by few people.

To assess the performance of local varieties for each trait, identify farmers who sow a particular variety. Then ask them to vote by raising hands on whether it performs well, satisfactorily, or poorly for a given trait, using this same line of questioning for each trait on the list. For example, for variety A, who sows it (count the number who raise their hands)? Then ask who thinks it is very good at withstanding drought (count those who raise their hands), who thinks it is satisfactory, and who thinks it is poor. Do the same for each trait on the list. Though long, this exercise provides a good assessment of local varieties' important traits. If the list is too long, queries can focus on traits identified as very important by most farmers in the previous exercise.

In this way, researchers can assess how local varieties supply the traits farmer demand and, thus, identify opportunities for improvement and possible trade-offs. For example, if a new variety performs well for one important trait but poorly for another important one, farmers can be asked whether this is acceptable.

To analyze the results of interviews, compare the traits mentioned across groups. Which were the traits everybody mentioned and which were not? Is there any pattern? Which were the traits mentioned by males and females? Which ones were mentioned by only one of these groups? In terms of ratings, what were the frequencies associated with each trait by group? These frequencies provide a quantitative estimate of the importance of the traits, which can be placed in simple tables with frequencies for cross-group comparisons. Are there differences? What is the consensus in terms of performance by trait? What are the trade-offs that farmers perceive in their local varieties (that is, for a particular variety, the simultaneous occurrence of a high frequency of a very good rating for a certain trait and a poor rating for another)?

The methods that rely on individuals are the same: 1) eliciting a set of traits that are important, 2) rating their importance, and 3) rating the performance of sown varieties with respect to the traits. These exercises are done individually, usually with a relatively large and representative random sample of farmers. Other socioeconomic information can be collected, so that afterwards researchers can relate the ratings to specific farmer conditions and make statistical inferences about their relationships. The individual approach requires more time and money.

Grain quality/type traits are very important for adoption. Here is it helpful to have samples of ears for farmers to categorize. Have, for example, ears with a range of textures to show farmers and have them rank them for desirability, being careful in a group setting to get the views of all present. When the most desirable type has been identified, then ask what it is that makes this type of grain so good. Possible responses are: storage quality, recovery rate of flour when milled, ease with which the grain can be shelled, color, depth of dent, or some trait associated with its performance as seed, etc.

7.3.3.3 Combining farmer groups and responses

- Identify groups of male or female farmers within a village or a region that share similar socioeconomic and biophysical conditions (farmer domains).
- Interview farmers that have been ranked by wealth and gender and ask them to classify their variety (or varieties) into one of the classes established above.
- Determine if particular varieties are associated with specific wealth classes among males and females.
- Use this two-way classification to develop varietal development goals and a target farmer group.

7.3.4 Evaluation of agricultural technologies

Identifying and understanding farmers' and scientists' perceptions of a technology (variety, or a management option that will lessen the impact of drought or low fertility) are fundamental to evaluating and improving it. Researchers need to identify the characteristics of a technology that are important to the farmer or scientist (such as crop varieties, fertility inputs, etc.) and ask whether these are considered benefits or costs. Another way is to ask informants to list the advantages and disadvantages of a technology. The frequencies associated with each answer should be calculated in general and for each farmer domain. The following is an example of responses from a group of farmers to a set of questions regarding their preferences for varieties:

Table 7.3.4.1. Disadvantages identified by maize farmers in Oaxaca, Mexico, for a particular variety.

Concern	Farmers' answers	Percentage	Cumulative
Yield	Low production	7.41	
	Low yield	18.52	
	Small cobs	7.41	
	Few rows	3.70	37.04
Storage	It rots	22.22	
	Not resistant to weevils	7.41	
	The cob rots	11.11	
	The grain rots	3.70	44.44
Abiotic stress	Tall plants (lodging)	14.81	14.81
Biotic stress	Attacked by pests	3.70	3.70
Total		**100**	

Table 7.3.4.2. Advantages identified by maize farmers in Oaxaca, Mexico, for a particular variety.

Concern	Farmers' answers	Percentage	Cumulative
Consumption	Good for making atole	3.87	
	Good quality	1.66	
	Color	8.29	
	Good for pasture	2.76	
	Good taste	12.15	
	Good for making tortillas	13.26	
	Good dough	1.66	
	Good for making tostadas	0.55	44.20
Yield	Thick grain	0.55	
	Produces cobs	0.55	
	High weight	12.71	
	Good production	1.66	
	Good yield	0.55	
	Good yield by volume	18.23	
	A lot of grain	0.55	34.80
Duration	Early	10.50	10.73
Sale	Sells well	2.21	2.26
Processing	Easy to shell	1.66	1.69
Adaptation	Well adapted	1.66	1.69
Abiotic stress	Withstands drought	1.10	
	Withstands cold	0.55	1.66
Biotic Stress	Withstands pests	0.55	
	Withstands weeds	0.55	1.10
Storage	Stores well	2.21	2.21
Total		**100.00**	

7.3.5 Learning from past technologies

Conditions and technologies used by farmers change. The challenge is how to use farmers' memories to learn about technological change. Identify informants of different age groups and gender, and identify a key and commonly shared moment in their lives (e.g., first marriage, outbreak of war, or a severe flood). Ask the informants about their current technology, and then about the one that served the same purpose at the key moment you have identified. Ask them to describe the current and past technologies, their advantages and disadvantages, and the reasons they abandoned or modified the past technology. If informants are in different age groups, this helps provide a sequence of events.

7.3.6 Farmers as experimenters

Why should farmers and scientists interact to carry out experiments? Scientists can help farmers improve their own experiments by providing some basic training and guidelines, and help farmers try and evaluate new technologies. Scientists also gain useful input about the technologies. In this case, the scientists should also provide basic training and guidelines for carrying out experiments, but the agenda is defined by the new technology.

Farmers' experiments may differ from those of scientists: Farmer's experiments usually lack a control treatment, several factors vary at once, they are usually unreplicated, and data collected are usually only impressions and observations.

Guidelines for developing experiments with farmers

- **Test only one factor at a time**. If there are several factors, test each one independently in a different field or section of the field

- **Emphasize the need for a control treatment**. Explain to the farmer the importance of a control treatment, to be able to interpret the results of the experiment. If there are several independent experiments in different fields, use the same control treatment to facilitate the comparisons and interpretations of results.

- **Emphasize the need to maintain all conditions, besides the experimental one, equal.** Jointly decide with the farmers what those conditions are, and agree on how they will be kept constant.

- **Establish the indicators and criteria to judge the outcome of the experiment**. Farmers and scientists may focus on different indicators to judge and interpret the outcome of an experiment and to assess the costs and benefits. The scientist should ask the farmer: what does s/he expect from the experiment? What elements would s/he focus on to judge it? Under what circumstances would the farmer judge one treatment to be better than the control? (And scientists should ask themselves the same questions!)

- **Replicate experiments among farms**: Farmers usually do not replicate their experiments and see replication as a waste of resources, because they lack the tools to utilize replications properly. They may also be unable to handle spatial variability in the plot. If replication of an experiment is considered important, do it across farms, even though experimental conditions may not be the same across farms. Convince farmers with replicates of the same experiment to agree on the conditions that should be maintained constant.

7.4 Conclusions

What should we be doing to ensure input by farmers into our plant breeding decisions? Two simple steps can be taken that could greatly improve our breeding goals, public awareness of what it is we are doing, and our chances of success:

- Be involved in interacting with groups of farmers selected as described above. Use samples of ears showing a wide diversity of characteristics to elicit opinions. Try to determine what farmers are already doing to handle risks associated with drought and low fertility. Identify management strategies that farmers use when drought threatens or actually occurs. Keep a written record of your interview procedure and the results.

- Involve farmers in your trials. Have them come to on-farm evaluation sites and to the experiment station and systematically collect their views and their preferences for the genotypes you are developing. Conduct a brief survey of those who come to determine who they actually are. Develop links with farmers who are keen to try out some of their selections on their own fields, and use this as a means of moving improved germplasm onto farmers' fields.

8 Literature

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66

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