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:

$$\mathrm{WUE} = (P_a - P_i) / (1.6 * (VP_i - VP_a)) \qquad [6]$$

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).

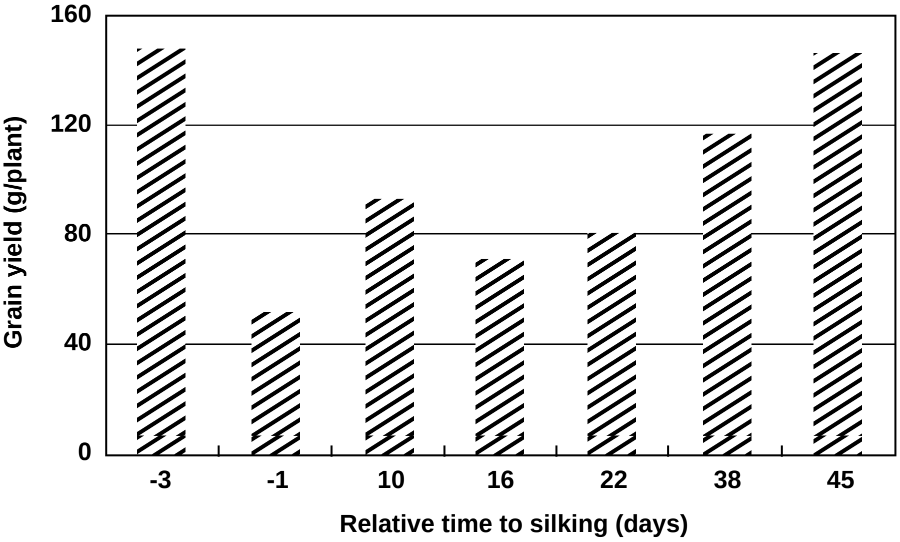


Fig. 2.1: Relationship between grain yield and timing of drought stress (Grant et al. 1989).

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

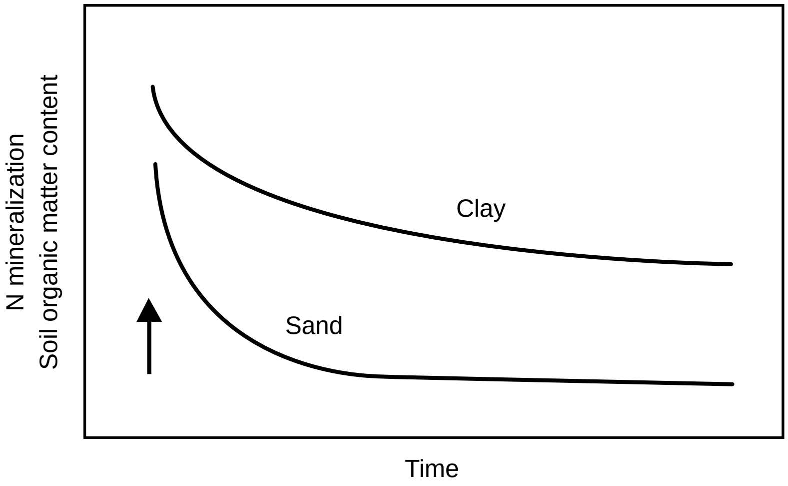
Most (95 to 99%) of the N in a field is not readily available to plants, because it is bound in the soil organic matter. Soil N is available to non-leguminous plants in the form of nitrate (NO_3) and ammonium (NH_4) ions. This pool of nitrate and ammonium is usually termed ‘**mineral N**’. Mineral N 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.



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).

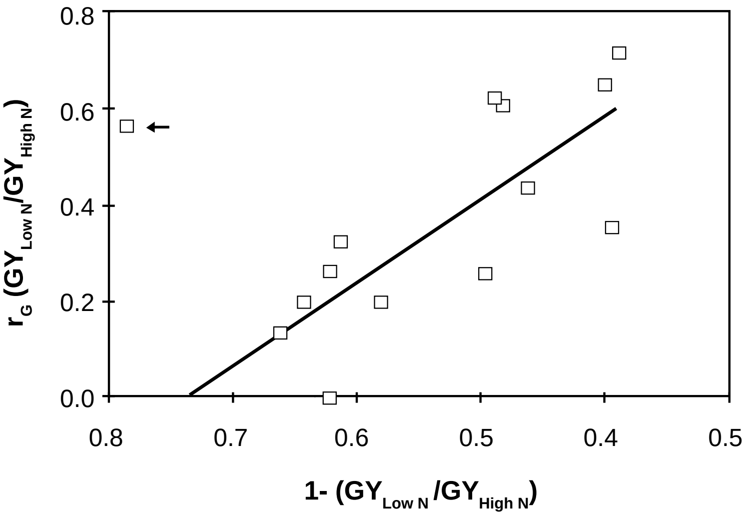


Fig. 2.3. Genetic correlation (r_G) between grain yields under low and high N vs. relative yield reduction under low N [1- ($GY_{Low\ N}/GY_{High\ N}$)] for 14 maize progeny trials evaluated at Poza Rica, México, between 1986 and 1995. Linear regression was $y = 1.19 - 1.58x$ ($R^2 = 0.62$, $P < 0.001$, $n = 13$) 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):

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

where T_{max} = daily maximum temperature

if $T_{max} > 34$ then $T_{max} = 34- 2.6*(T_{max}-34)$

if $T_{max} > 44$ then $T_{max} = 34- 2.6*(44-34) = 8$

T_{min} = daily minimum temperature

if $T_{min} < 8$ then $T_{min} = 8$

$\sum$ = 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:

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

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.

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

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

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

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.

	Pan placed in short green cropped area				Pan placed in dry fallow area			
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 (Vol %)	Permanent wilting point (Vol %)	Plant-available water (mm/10 cm depth)	Bulk density (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.