4.1 Field Screening for Drought Tolerance – Principles and Illustrations

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Abstract

Establishing a screening procedure for genetic differences in drought tolerance involves 1) practical decisions on the objectives of such a screening program, 2) the selection of environment(s) and stress occurrence(s) to be targeted in the program, and 3) the design and operation of field physical facilities and experimental methods to apply a uniform, repeatable drought stress. This paper considers these points from a conceptual and a practical viewpoint.

Drought tolerance can be approached on various plant organizational levels, from crop yield stability under stress, through responses to stress indicative of tolerance, to the biological mechanisms that underlie these responses, to the genes and alleles governing the presence or expression of the responses/mechanisms. Defining stress tolerance at each level has specific advantages and disadvantages for designing a field-screening program. Work on pearl millet has mainly focused on the crop tolerance response level, targeting the relative ability of genotypes to maintain grain numbers per panicle and seed filling in terminal stress environments.

Target environments and target stress occurrences for a screening program must be established from the analysis of historical climate data. Water budgeting is probably the minimum level, but opportunities to use crop simulation modeling for this purpose are improving. Establishing screening systems with environmental conditions representative of the target environment is difficult, involving a major tradeoff between providing representative daylength, vapor pressure, and temperature conditions, and easily managing soil water/rainfall. In contrast, duplicating target environment moisture patterns in non-target environments is easier, but G × E effects can be a problem.

The effectiveness of a drought screening procedure is best measured by the genetic heritabilities achieved for target traits, whether the focus is

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conventional or marker-assisted plant breeding. Managing drought screening nurseries therefore requires careful analysis of likely sources of nongenetic variation among plots, replications, and repeated experiments, and seeing that these are minimized. These include 1) the choice of site for screening, 2) the physical management of both water-related and non water-related sources of variation in crop growth within and across experiments, 3) the choice of experimental design and the effective use of blocking to remove expected sources of nonmanageable variation, and 4) the efficient collection and management of data. These considerations are illustrated here with examples from the pearl millet drought screening system used at ICRISAT.

Introduction

Accurate field phenotyping of mapping populations, for traits as complex as drought tolerance, is almost certainly the limiting factor in our ability to detect and evaluate molecular markers for such traits. The creation and genotyping of mapping populations is often the more expensive part of the overall effort, but its ultimate success depends much more on the effectiveness of the phenotyping procedure in detecting repeatable, highly heritable differences among recombinant lines, that permit the identification of robust quantitative trait loci (QTL). Drought tolerance is a particularly difficult topic for molecular mapping as it is not possible to define or measure tolerance with the same clarity or precision as it is for disease resistance or for morphological or physiological traits, nor is it easy to manage experimental drought environments with a high level of control and repeatability. Therefore, extra effort is needed in the conceptualization, design, and management of phenotyping programs for drought tolerance, to maximize the chances of identifying QTL that will be useful in the future improvement of tolerance in the target crop and in the target environment. This paper reviews some of these considerations in 1) developing a functional definition of drought tolerance to use in a screening program, 2) designing screening procedures to focus effectively on the target environment and its major stress problem(s), and 3) managing the screening experiments to minimize problems in detecting heritable differences in tolerance. General considerations will be illustrated by examples from the screening program for terminal drought tolerance in pearl millet [*Pennisetum glaucum* (L.) R. Br.] at ICRISAT.

Defining Drought Tolerance

Drought tolerance has been defined in many ways in the past; but not all of these are likely to be equally useful for a program with the ultimate goal of genetic improvement of crop yield, or the stability of crop yield, under drought stress. It is possible to group various approaches to defining stress tolerance into the following four hierarchical classes, each of which has its own implications for use in a screening program.

A stable grain yield despite the occurrence of stress. Although a more stable yield is the ultimate objective of stress research, and while the presence of desirable traits, mechanisms, or QTL should result in a more stable yield, yield under stress is probably too complex a phenomenon to use as a variable for evaluating stress tolerance per se, as it represents genotype response to the total of the environmental factors to which it has been exposed over the course of the entire season. In addition, grain yield has predictably very large environmental (E) and genotype × environmental (G × E) effects, and consequent modest across-environment heritabilities, which reduce its value as a screening/selection criterion.

The maintenance of normal developmental and growth processes under stress (such as maintenance of normal water status, developmental events, and leaf area). Focusing screening on such processes has the advantage of better focusing on unambiguous expressions of field resistance/susceptibility, rather than on yield itself. At the same time, it is often relatively straightforward to link the maintenance of normal growth processes under stress to more stable yields. On the other hand, the field quantification of such responses may be considerably more demanding than quantifying yield differences, and their expression, and therefore heritability, may also be affected by G × E interactions.

The biological mechanisms underlying these favorable responses under stress. Associating drought tolerance with the existence or expression of specific biological mechanisms (e.g. maintenance of plant water status or cell turgor) can help greatly in defining the focus of field or controlled environment screening and in establishing screening protocols which allow better management of E and G × E influences. However, a focus on underlying mechanisms is likely to be at the cost of the linkages to final grain yield, and to increased measurement costs, thereby complicating conventional and molecular breeding for tolerance.

The loci or alleles that underlie these biological mechanisms. Focusing on genes coding for basic mechanisms can (theoretically at least) greatly simplify the problem of breeding for drought tolerance to one of simply selecting for established DNA markers, without the effects of E and G × E interactions that complicate phenotypic selection. However, it is very likely that adaptive responses to stress are multigenic, and that the expression and consequences (if not the presence) of QTL for stress tolerance are still subject to G × E influences. More experience with QTL as selection criteria for stress tolerance is needed before it will be possible to confidently equate stress tolerance to the presence of selected QTL.

A useful, applicable criterion for stress tolerance, and ultimately a useful selection criterion, should have several attributes, which may not always be fully compatible in a single definition:

- There must be a clear, strong linkage between drought tolerance and higher or more stable grain yield in the target stress environment.
- The across-stress-environment heritability of tolerance should ideally be higher than that of grain yield itself.
- The expression of tolerance must be readily measurable, with adequate replication in both time and space, of the numbers of genotypes necessary in contemporary phenotyping/breeding programs.

These requirements tend to favor specific whole plant or crop responses to stress that are clearly linked to yield maintenance, and which can be readily and repeatedly measured on large numbers of genotypes, such as the anthesis-silking interval used as an indicator of differential susceptibility to stress at flowering in maize (Bolanos and Edmeades 1996). More basic physiological and chemical mechanisms, by and large, are not sufficiently strongly linked to yield maintenance under stress, and have major sampling and measurement limitations for large populations, that make them less attractive as functional definitions of drought tolerance.

Defining Terminal Stress Tolerance in Pearl Millet

An analysis of factors associated with differential ability to maintain grain yields under terminal stress in pearl millet has led us to identify panicle harvest index or PNHI (the ratio of grain to total panicle weight, on a plot basis – line 1, Table 4.1.1) as an indicator of genetic tolerance/ susceptibility to such stress (Fussell et al. 1991). Stress beginning at different times during the flowering

Table 4.1.1. Effects of increasing severity of terminal drought stress on pearl millet panicle yield components and panicle harvest index (hypothetical data).

Drought severity and time of onset	Rachis, glumes, etc. (g)	Grains per panicle (no.)	Single grain mass (g)	Total grain mass (g)	Total panicle mass (g)	Panicle harvest index (%)
Non-stress	5.0	1500	.0100	15.0	20.0	75
Mild, Late onset	5.0	1500	.0085 (–15%)	12.8	17.8	72
Moderate, late onset	5.0	1500	.0070 (–30%)	10.5	15.5	68
Moderate, mid onset	5.0	1275 (–15%)	.0070 (–30%)	8.9	13.9	64
Severe, mid onset	5.0	1275 (–15%)	.0055 (–45%)	7.0	12.0	58
Severe, early onset	5.0	1050 (–30%)	.0055 (–45%)	5.8	10.8	53
Severe, pre-flowering onset	3.5	600 (–30%)	.0040 (–60%)	2.4 (–60%)	5.9	42

and grain filling periods affects the various panicle yield components formed during these periods in predictable ways. For example, a stress beginning late in the grain filling period will affect mainly individual grain mass; a 15% reduction in individual grain mass will reduce total panicle grain mass from 15.0 to 12.8 g, total panicle mass from 20.0 to 17.8 g, and PNHI from 75% to 72% (Table 4.1.1, line 2). Similarly, a 30% reduction in individual grain mass will reduce PNHI from 75 to 68% (Table 4.1.1, line 3). A stress beginning earlier will reduce both grain number and individual grain mass, with greater effects on PNHI (Table 4.1.1, lines 3, 4, and 5). In this fashion, PNHI is a simple but effective measurement for quantifying the known effects of stress during flowering and grain filling.

Different levels of genetic tolerance, expressed as differential ability to maintain both grain numbers and grain filling under stress, are effectively captured by differences in PNHI (Table 4.1.2). For example, a tolerant genotype will more effectively maintain both grain number and individual grain mass, than will an intermediate or susceptible one (compare lines 3, 4,

Table 4.1.2. Consequences of different levels of terminal stress tolerance on panicle components and panicle harvest index (hypothetical data).

Genotype level of tolerance	Rachis, glumes, etc. (g)	Grains per panicle (no.)	Single grain mass (g)	Total grain mass (g)	Total panicle mass (g)	Panicle harvest index (%)
Non-stress	5.0	1500	.0100	15.0	20.0	75
Escape – early flowering	5.0	1500	.0085 (–15%)	12.8	17.8	72
Tolerant	5.0	1350 (–10%)	.0085 (–15%)	11.5	16.5	70
Inter- Mediate	5.0	1200 (–20%)	.0070 (–30%)	8.4	13.4	6
Susceptible	5.0	1200 (–20%)	.0050 (–50%)	6.0	11.0	5

and 5 in Table 4.1.2), which is clearly reflected in the differences in PNHI. Because PNHI integrates the effects of stress on both grain number and grain filling, it is less subject to compensatory tradeoffs between individual yield components, and is better related to yield-based estimates of tolerance/ susceptibility to terminal drought stress than are the individual components. Panicle harvest index is, however, influenced by differences in drought escape (i.e. by differences in the severity of stress actually experienced by different genotypes), so valid comparisons can be made only between genotypes with similar flowering times.

Panicle harvest index has been successfully evaluated as a selection criterion for terminal stress tolerance in pearl millet in both variety and hybrid parent breeding (Bidinger et al. 2000) and it is currently being used as one of the traits for which QTL are being identified from a mapping population made from parents that differ in the ability to maintain PNHI under stress. PNHI, however, is readily and inexpensively measured in field experiments, and can be readily used as a direct selection criterion. The main potential benefit to identifying QTL for PNHI will be in allowing rapid, marker-assisted backcross transfer of improved tolerance of terminal stress to otherwise elite lines and varieties, without the requirement for extensive field screening.

Selection of a Screening Environment/Method

Experimental procedures to screen for drought tolerance, however this is defined, need to be effective in identifying heritable genetic variation *for the specific target environment and the target stress (es) in this environment.* They thus need to reliably provide stresses of the timing, severity, and duration characteristic of those stresses common in the target environment. Quantifying the nature of the stress (es) in the target environment requires an analysis of long term climatic data; using, as a minimum, a water balance model approach which integrates rainfall, plant-available water in the soil, potential evaporative demand, and crop coefficient (Frere and Popov 1979). Crop simulation modeling can provide a much more rigorous analysis; if an appropriate crop model and long term weather data sets are available (Muchow et al. 1999). The better the description of the variation in the occurrence of stress in the target environment, the better targeted the screening is likely to be.

Screening environments can be either natural growing environments, chosen/managed to maximize the frequency of stress under natural environmental conditions, or specially managed stress environments in which the emphasis is primarily on a providing a controlled, repeatable stress. Whether or not the screening environment needs to exactly duplicate the overall target environment depends partly on the way in which drought tolerance is to be assessed. If the screening is targeting a yield-based definition of tolerance, then the environmental conditions of the screening environment which affect yield need to duplicate those of the target environment. For example, if daylength in the target and screening environments differs to a degree sufficient to affect phenology, then drought escape, which can play a large role in the determination of yield under stress, will operate differently in the screening and target environments. Under such conditions, it is better to use a variant of the natural target environment (rain shadow sites, shallow soil fields, late sowing) where stress is likely. However, if the intent is to evaluate more basic stress responses or tolerance mechanisms, it may be feasible to use non-natural growing environments such as a dry season or more arid locations where the occurrence and severity of stress can be controlled though management of irrigation or sowing date.

Managed stress environments have definite advantages in terms of control and repeatability of stress, with consequent advantages in control of G × E interactions and improved heritabilities of tolerance-related

observations. Managed stress environments can also be used to exploit repeatable genotype × stress interactions to improve specific adaptation to defined stresses in the target environment. There are two options for using managed stress environments: 1) artificially creating stress in a normal growing season, and 2) managing water availability in the dry season. The first option has the definite advantage of avoiding genotype × season interactions, which can affect genotype response to stress, but excluding water to create stress in a normal growing season can be costly/difficult. Using rainout shelters, covering the surface of soils to encourage runoff, etc. are feasible for small, critical experiments, but less so for large-scale screening exercises for most field crops. Managing water in the dry season or a dry location has the advantages of scale, reliability, and economy of screening, but may require verifying that the expression of tolerance is not affected by genotype × season interactions. Most field screening is done under managed stress environments, but there is often inadequate assessment of the repeatability of genetic differences observed in the dry season, in the target environment itself.

Whatever the screening environment selected, the screening protocol designed needs to achieve the following objectives:

Application of a Uniform Moisture Stress

Unless all genotypes in the screen are exposed to a similar stress, the measured differences among them are as or more likely to reflect differences in stress experienced, than differences in stress tolerance/susceptibility (Blum, this volume). The screening procedure thus must assure uniform water application rates, uniform soil water storage/plant-available water content, and a uniform rate of potential water use. Some of this is a matter of good experimental management, but choice of field, especially soil texture and depth, and design of water application systems can also make large differences. Sprinkler irrigation, for example, is convenient but seldom uniform.

Application of Repeatable Moisture Stress

Uniformity across experiments is as critical as uniformity within experiments in obtaining broad sense heritabilities of sufficient magnitude to use in either direct selection for tolerance, or in the identification of tolerance QTL. Repeatability over experiments requires a screening environment with stable potential evaporation, a regular, dedicated field screening facility, and well

established field and crop management systems, to minimize G × E interaction effects on tolerance expression.

Effective Differentiation between Genotypes

To effectively distinguish differences among genotypes requires that stress is of a sufficient severity to obtain statistically significant differences among genotypes for the measurements of stress tolerance to be made, but not so severe that genotype differences are expressed. It also requires that differences among genotypes due to differential stress exposure (stress escape), rather than to differential stress tolerance/susceptibility, be minimized. Achieving both of these objectives will require some initial experimentation; particularly where there are significant differences in phenology among test materials.

Screening Environment for Terminal Stress Tolerance in Pearl Millet

The main growing area in the northwest (NW) Indian states of Rajasthan, Gujarat, and Haryana is the target environment for ICRISAT work on stress tolerance in pearl millet. This area has a short (75 to 90 day) growing season with a total seasonal rainfall between 250 and 500 mm, in a generally arid to dry semi-arid climate. Soils are mainly sandy, with low to moderate levels of plant-available water content. Growing season temperatures (mean maximum ~33° and mean minimum ~25°) and potential evaporation rates are high ($\geq$ 6 mm day^{-1}). An analysis of the frequency of occurrence of drought stress, based on a five-day soil water budget, for a transect across central and western Rajasthan indicated that post-flowering stress, either alone or in combination with preflowering stress, is a very common feature of the environment (Table 4.1.3, van Oosterom et al. 1996). In the two drier sites (Bikaner and Barmer) terminal drought occurred between 75 and 80% of the years: between 15 and 30% percent of the years alone, and in 50–60% of the years in combination with preflowering drought. ICRISAT millet research has thus focused on terminal drought tolerance, as terminal stress is clearly a common feature of the target environment and is the most damaging to grain yield, as the crop has few adjustment mechanisms available to it in contrast to the situation with preflowering drought stress (Mahalakshmi et al. 1987).

Table 4.1.3. Distribution of years with various combinations of severe pre- and post-flowering drought stress at four locations in Rajasthan. Values in parentheses are frequencies (%). Results are based on water balance studies, using long-term daily rainfall data (van Oosterom et. al. 1996).

	Severe drought stress class			
Preflowering	No	Yes	No	Yes
Postflowering	No	No	Yes	Yes
Ajmer	63 (72)	3 (3)	19 (22)	2 (2)
Jodhpur	26 (31)	8 (10)	33 (39)	17(20)
Bikaner	6 (7)	13 (16)	13 (16)	51(61)
Barmer	5 (9)	7 (13)	16 (29)	28(50)

Managed irrigation has been used during the dry season at Patancheru for the majority of the screening work, although key trials are regularly planted in the target area as well. The main reason for this is the requirement for very high-quality trial management on a large scale (4–6 ha yr^{-1}), which has been difficult to achieve on collaborators' research stations in NW India. The use of irrigation in the dry season allows effective (and repeatable) management of the timing and severity of the stress. Temperatures and vapor pressure deficits during March/April, when the stress is applied, are representative of those during drought periods in NW India. We know however, that we have genotype × season interactions for actual grain yield with landrace material from NW India, possibly because of differences in early season temperatures, and differences in day length, between the dry season at Patancheru (17° N) and the normal season in NW India (23–28° N). We believe that genotype × season interaction for PNHI and its components is not a serious problem, where flowering, and hence drought escape, is not influenced by genotype × season interactions for phenology.

Management of Screening Nurseries

Effective screening for genotype differences in drought tolerance/susceptibility requires a high degree of care in the design and management of the trials to obtain precise data and to maximize the heritability of the selected measurements of drought tolerance. This is particularly critical in field experiments designed to identify QTL for tolerance, as the strength of QTL for target traits depends directly on the heritability of these traits achieved in

the experiment. The more the measure of drought tolerance is influenced by local environmental variation, the greater is the need to control such variation. Effective management of experiments to control variation requires a number of components:

Field Screening Facility

The screening facility must be capable of applying a uniform stress to a large set of genotypes. This means that the soil of the field must have uniform plant-available water content, that the irrigation system chosen must be able to apply water uniformly, and that the location of the field be such that it has a spatially uniform rate of potential evapotranspiration (ET). Any deviation from these three requirements (such as variation in soil texture or depth, nonuniform irrigation water application, or inadequate fetch or local windbreaks) will result in gradients (at best) or nonlinear heterogeneity (at worst) in the timing or severity of the stress applied. In addition, it is necessary to be able to repeat stress environment (timing, severity, and duration) over experiments, to confirm genotype differences in tolerance and to maximize across-environment heritability of tolerance estimates. To do this, it is generally necessary to have a dedicated field for screening, in a stable water use environment, and to use consistent, well-established crop, soil, and water management practices.

Statistical Design

Despite maximum care in the choice and management of a field screening facility, there will still be experimental error: soils are not commonly uniform in depth or texture; most irrigation systems have inherent gradients in water application. It is necessary to understand the sources of experimental error in a screening procedure, and to use appropriate statistical designs and field blocking to remove as much of the known sources of error as possible. Unbalanced lattice or alpha designs, which allow a high degree of blocking within replication, can be very useful to adjust for both primary (replication) and secondary (within-replication blocks) gradients in soil water holding capacity, water application patterns, etc. Small blocks also provide greater flexibility in field layout that larger replications do not, and provide useful ability to adjust for the effects of time in the collection of data where this is a major confounding factor (plant water potential or water content).

Field/Crop Management

In addition to variation in factors affecting water availability, differences in crop growth prior to the application of stress are often major confounding factors in the assessment of stress tolerance, as these result in differences in ability to access soil water, in canopy transpiration rate, or in inter-plot competition. It is necessary to improve field management practices to eliminate, as much as possible, differences in plant stands, in fertilizer application rate, in pre-stress water application and drainage, and in pest and disease incidence. Management of the final irrigation prior to initiating the stress is a particularly critical factor. This should be designed to completely fill the soil profile to eliminate differences in soil water storage due to the effects of previous irrigation, or differences in water use among genotypes, and to then rapidly drain excess water to prevent local waterlogging. Finally, the experimenter should be prepared to learn from past problems and to adjust management practices to minimize these.

Data Management

Finally, the screening system needs to be organized to record, manage, and verify large volumes of data from screening experiments. Data collection should be done electronically wherever possible; simple and inexpensive equipment is available for recording scores, weights, and measures; in the case of weights, these can be directly linked to electronic balances, so all that needs to actually be entered is the plot identity. This reduces both time and errors in recording data. Also, quick and efficient procedures for checking the completeness and accuracy of data are easy to establish with modern spreadsheet/analytical software, which will detect outliers and missing plots, and calculate means, ranges, and basic statistics. Finally, linking spreadsheets to analysis packages can allow the scientist rapid access to analyzed data to check heritabilities of measurements and means for control entries.

Management of Pearl Millet Screening Nurseries

Field Screening Facility

For field screening, only a designated six hectare field with a shallow and relatively uniform soil profile is used, which contains enough plant-available water for about 6 days of full ET during April, when pan evaporation rates

average 8–10 mm day^{-1}. As a part of the development of this field for surface irrigation, the A and B horizons of the original soil (50–75 cm depth) were removed, the gravelly subsoil material graded to a uniform slope of 1.5%, and the surface soil spread evenly over the graded subsoil. Thus the major source of heterogeneity in the original field – the variable depth of soil to the C horizon, and the consequent variable amount of plant-available water – has been largely removed.

Sprinkler irrigation is used to supply water to the crop before flowering, adjusting the amounts of water applied to meet increases in transpiration demand as the season progresses, as millet is sensitive to low soil oxygen tensions that occur following surface (furrow) irrigation during cooler times of the year. Sprinkler lines are placed 14.4 m (24 crop rows) apart, with each sprinkler line in the center of 4 border rows, so that leakage from the sprinkler lines does not affect test plots. Final irrigation before the onset of the stress is done by furrow, to completely fill the soil profile.

The time of planting of the nurseries is standardized to have the crop flower and fill grain during the period of maximum evaporative demand, and irrigation is managed to achieve a 50–60 % reduction in yield for a severe stress and a 30–40% reduction for a moderate stress. Standard crop management procedures (described below) are followed to obtain uniform preflowering crop growth and initiate the stress (es) at fixed crop developmental stage(s). This latter is necessary as differences in temperatures during the earlier, cooler part of the growing season can affect time to flowering, even though a common planting time across years assures a similar daylength each year.

Statistical Design

Incomplete block or alpha designs are generally used in the majority of screening experiments, to provide for as much adjustment capability to local variation in stress intensity as possible. Small blocks of between 6 and 9 plots are used (18–27 m^2/block), with the total number of blocks variable, depending upon the numbers of entries in the trial. It is generally found that the effect of such blocking is statistically significant, despite the general precautions taken in managing experimental crops.

The sprinkler irrigation system used provides standard 20 row experimental 90-m long strips between the lines (Fig. 4.1.1). We replicate along the 90-m axis to adjust for differences in water application due either to decreasing pressure in the sprinkler line before flowering, or to differences in

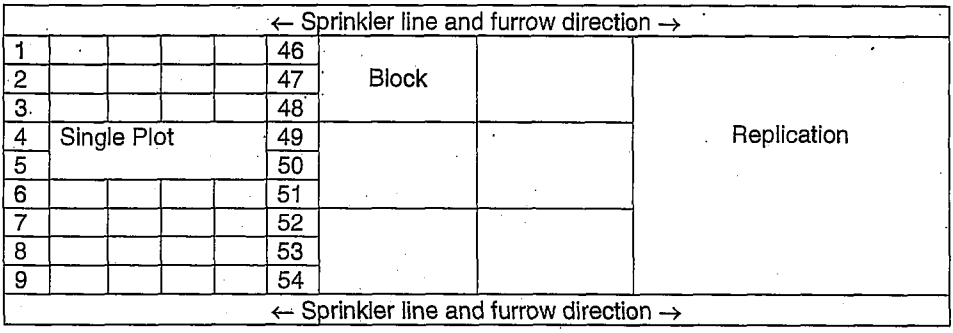

Figure 4.1.1. A example of plot, block, and replication arrangement within one experimental strip between two irrigation lines, from a phenotyping experiment conducted on 162 mapped F2 - derived F4 lines. Each block consists of 9 plots (3 plots wide × 3 plots long); each replication consists of 18 such blocks (6 blocks per strip × 3 strips); and one stress environment consists of 3 replications (across 3 such strips).

time for water infiltration along the 90-m axis during the final prestress surface irrigation. The blocks are then arranged at right angles to the sprinkler lines (and the 90-m axis) to adjust for differences in water application between the sprinkler lines before the stress, or to miscellaneous local variation (Fig. 4.1.1). The ICRISAT statistician is currently evaluating the effectiveness of spatial adjustment techniques to further reduce effects of both inherent and management-induced variation.

Field/Crop Management

A number of ways to improve the uniformity of crop growth prior to the initiation of the stress have been learned by experience. 1) The field is land planed every 2 to 3 years to remove local surface irregularities that result in collection of excess irrigation water and reduced crop growth. 2) Fertilizer is banded into the ridges with a precision applicator, rather than broadcasting it, to assure that all seedlings have equal access to nutrients. 3) Light sprinkler irrigation is provided prior to sowing, to moisten the surface soil and improve control over the depth of seed placement. 4) Oversowing is done with a precision planter and seedlings thinned about 10 days after emergence to achieve uniform plant stands. 5) Sprinkler irrigation is used in the early crop stages, rather than furrow irrigation, to prevent excess water application and reduced crop growth. 6) Sprinkler irrigation is provided at the time of

secondary root initiation to assure that these roots penetrate the soil rapidly and completely. 7) Weed management is practiced during the entire year in the screening field to prevent the buildup of weed seed, and cultivation is done early and as often as necessary to remove weed seedlings in early stages before they can establish. 8) Prophylactic pest and disease control is applied whenever a problem is suspected (for example a soil insecticide is banded with the seed to control wireworms, when following a groundnut crop).

At the time of initiation of the stress, furrow irrigation is used to be sure that the full soil profile is wetted. The furrows are filled rapidly, one strip at a time, to have a sufficient head of water for this purpose. Water is held in the furrows for 4 hours and then drained rapidly to prevent waterlogging. All irrigation operations are managed by the researchers themselves to assure that irrigation is done as precisely and uniformly as possible.

Data Management

Hand-held data collection devices (Tandy portable computers and Omnidata polycorders) are used to record all information taken in the field or the laboratory. This includes flowering dates, plant and panicle counts, plot scores for various criteria, and outputs from instruments without microprocessor storage. All of the balances are linked to one or more of the same instruments so that the weight is automatically recorded by depressing the enter key, following the manual entry of the plot numbers. For this purpose plot numbers are never repeated within a season, so that it is not necessary to identify the experiment, location, etc. in the data entry.

Data are downloaded to a personal computer twice a day (noon and evening) to prevent loss of data stored on data collection devices. Because unique plot numbers are used, it is easy to sort data (such as grain weights) in a spreadsheet from more than one experiment and, by ordering plots in ascending order, to quickly determine if any plots have been missed or if there are any duplicate plot numbers entered. Means and standard deviations are calculated for all variables with either Excel or SAS to establish expected ranges of data values, and possible outliers are searched for using the delete and print option in SAS.

Rapid, same day analysis of data can be done if required, as all variable names are standardized, transformation routines to calculated derived variables, conversion of plot values to unit area values, etc. are standard, and

analysis models/statements written for each experiment. Finally one staff member handles all data analysis and archiving, after the technicians check it for missing values.

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