Breeding Rice for Drought-Prone Environments

Edited by K.S. Fischer, R. Lafitte, S. Fukai,
G. Atlin, and B. Hardy

IRRI

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2003
IRRI

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Mailing address: DAPO Box 7777, Metro Manila, Philippines
Phone: +63 (2) 580-5600, 845-0563, 844-3351 to 53
Fax: +63 (2) 580-5699, 891-1292, 845-0606
Email: irri@cgiar.org
Home page: www.irri.org
Riceweb: www.riceweb.org
Riceworld: www.riceworld.org
Courier address: Suite 1009, Pacific Bank Building
6776 Ayala Avenue, Makati City, Philippines
Tel. (63-2) 891-1236, 891-1174, 891-1258, 891-1303

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ACKNOWLEDGMENTS

The"Workshop on Field Screening for Drought Tolerance in Rice" held in 2001 at ICRISAT highlighted the need for a practical guide for the breeding of rice for drought-prone environments. Some of the material used in this manual was reported at the workshop. In developing this manual, we were influenced by the focused and practical approach of Marianne Bänziger and the CIMMYT team in their manual *Maize Breeding for Drought and Nitrogen Stress Tolerance* and we have adapted some material from them. Thus, we acknowledge the generosity of CIMMYT and ICRISAT in permitting the use of these materials.

John O'Toole has been working for the improvement of drought tolerance in rice for all of his professional career. As a member of The Rockefeller Foundation, he has developed a large network of scientists focused on improving rice varieties for farmers in unfavorable environments. The Rockefeller Foundation provided the financial support for this manual.

Many authors and practitioners have contributed to the manual and they are recognized in the authorship of the different sections. Others helped in various stages of compiling the manual: Bill Hardy harmonized the styles of many contributors into clear language, Lucy Gamel facilitated the exchange of each successive version, and Jaya Basnayake and Jaquie Mitchell edited figures and tables.

We thank those who provided photos for use in this manual. The photos should not be copied or used without their permission.

The final quality of the manual is due to the excellent comments by Abraham Blum and Graham Wilson, whose professional careers have been dedicated to crop improvement in variable environments.

ACRONYMS, ABBREVIATIONS, AND ICONS

See

Early generation testing

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IRRISTAT

Evaluations with farmers

Parental selection

Multiple-environment trials

ASI	anthesis-to-silking interval
CIMMYT	International Maize and Wheat Improvement Center
CV	coefficient of variation
DH	doubled-haploid
DM	dry matter
DRI	drought response index
G	genotype
GEI	genotype × environment interactions
GL	genotype × location
GLD	green leaf duration
GLY	genotype × location × year
GY	genotype × year
H	heritability
HI	harvest index
IRIS	International Rice Information System
IRT	infrared thermometer
LSD	least significant difference
LWP	leaf water potential
MAS	marker-assisted selection
METs	multiple-environment trials
MSE	mean square error
NILs	near-isogenic lines
OA	osmotic adjustment
PHI	panicle harvest index
QTL	quantitative trait loci
RCB	randomized complete block
RGA	rapid generation advance
RILs	recombinant inbred lines
RUE	radiation-use efficiency
RWC	relative water content
S	supply of water
SE	selection environments
SSD	single-seed descent
T	water transpired
TPE	target population of environments
WUE	water-use efficiency
Y	year

SECTION

1

About this manual

K.S. Fischer, G. Atlin, A. Blum, S. Fukai, R. Lafitte, and D. Mackill

Drought is a major problem for rice, but most improved rice varieties are susceptible to drought.

Why a manual?

A large portion of the world's poor farm in rainfed systems where the water supply is unpredictable and droughts are common. In Asia, about 50% of all the rice land is rainfed and, although rice yields in irrigated systems have doubled and tripled over the past 30 years, only modest gains have occurred in rainfed rice systems. In part, this is because of the difficulty in improving rice varieties for environments that are heterogeneous and variable, and in part because there has been little effort to breed rice for drought tolerance.

Information available for other cereals (for example, maize, Bänziger et al 2000) and for wheat and the limited or circumstantial evidence available for rice indicate that we can now breed varieties that have improved yield under drought and produce high yields in the good seasons.

This manual aims to help plant breeders develop such varieties.

While the manual focuses on drought tolerance, this must be integrated with the mainstream breeding program that also deals with agronomic adaptation, grain quality, and pest and disease resistance. Mackill et al (1996) have written a guide to the overall improvement of rice for rainfed conditions. This manual should be seen as an amplification of and updating of the section on drought tolerance in that book.

Because final proof of many approaches for breeding drought-tolerant rice is not yet available, and because some aspects may not work in all environments and germplasm, we recommend that you use this manual with caution. Test the suggested approaches and only implement them on a large scale if they are effective and realistic for your own situation.

What is new in the approach for breeding for drought-prone environments?

Generally, breeding methods for rainfed rice have been influenced strongly by the experience in irrigated rice, where the crop is usually grown under stress-free conditions and yields in farmers' fields approach those on experiment stations. Most conventional plant breeders in rainfed systems use the early screening phase to select for traits such as height, maturity, plant type, pest tolerance, and grain quality, often under well-watered conditions on research stations. Only in the advanced testing stage, when relatively few genotypes remain, are entries evaluated under the stress conditions of farmers' fields. The outcome is often a variety that performs well under well-watered conditions and poorly under stress.

In contrast to this conventional approach, growing evidence indicates that varieties can be developed for improved yield under drought stress yet respond to well-watered conditions if there is early selection for yield in both environments. Thus, a key requirement for changing the breeding approach is to know the target environment in which the improved varieties are to be grown and ensure that the testing environments represent that environment. This issue is examined in Section 2.

There are several reasons for plant breeders' apprehension about selection under drought stress. The drought environment where selection and testing work are done is often spatially variable. This variation (and uncertainty of outcome) raises such practical questions as

- Should selection be done indirectly in high-yield environments (where genetic variance is usually maximized) or directly in the presence of the relevant stress or in both?
- Is it biologically possible to develop cultivars that combine stress tolerance with responsiveness to favorable conditions?
- Can selection conducted under controlled conditions (usually on-station) result in improved performance on-farm in marginal or stress environments?

These concerns are examined in Section 3, where the manual outlines a breeding approach, based on direct selection for grain yield, that maximizes selection progress for the target environment.

Which drought traits are useful?

Although progress can be made by selection for yield in the target environment, using physiological traits that are associated with drought tolerance can hasten that progress. Several putative traits might affect the response of the plant to drought, but we have firm evidence that only a few contribute to yield in the target environment. It is these traits of known value that, combined with selection for yield per se, can improve the plant breeding process either in parental selection or in the screening of segregating material. In Section 4, we provide a basic physiological understanding of the yield of rice under water stress, and discuss the most useful traits for breeding and the breeding approach for indirect selection for drought tolerance.

Who is using these approaches?

A growing number of research programs are using these approaches for breeding for drought tolerance in rice (and in other crops). The last section (Section 5) contains case studies of how others are implementing these new approaches in their unique environments. We recognize that much more experience and research are needed on many topics. For example, there is a large effort to apply molecular techniques to the improvement of drought tolerance in rice. But these have not yet been used routinely in conventional breeding programs (although the future holds promise) and are not included in this version of the manual.

As additional information becomes available and new techniques are tested, revisions to this manual may be needed.

How to use the manual

There are two levels of information in the main sections of the manual: practice and theory. The information on practice is brief and to the point and provides guiding principles to assist you in implementation. It is highlighted. More details on important areas are provided in the theory behind the practice in each section. This material is not highlighted.

Notes

Authors' addresses: K.S. Fischer and S. Fukai, University of Queensland, Brisbane, Australia; G. Atlin and R. Lafitte, International Rice Research Institute, DAPO Box 7777, Metro Manila, Philippines; A. Blum, PO Box 16246, Tel Aviv 61162, Israel.

SECTION

2

Know your target environment

K.S. Fischer, S. Fukai, R. Lafitte, and G. McLaren

Successful breeding programs must define the target environment.

Our aim is to improve the performance of rice varieties grown under drought. The performance, or the phenotype, depends on the genotype and the production environment.

To improve crop performance in a given environment, it is necessary to (1) know and understand the target environment and (2) define the strategy for changing the genotype (variety) by plant breeding (Cooper and Byth 1996).

What is the target environment?

The crop is grown in a complex set of socio-physical and biological environments that determine the performance and adoption of the preferred variety. Thus, there is no one environment even on the same farm; rather, there are several environments that will change from year to year and from field to field. We refer to these as “the target population of environments” (TPE). Each breeding program must clearly define the TPE for which it is developing varieties.

Thus, a TPE is the set of all environments, fields, and seasons in which improved varieties are expected to do well. These environments vary in predictable ways such as annual rainfall patterns, toposequence, soil type, and cultural practices and in unpredictable ways such as random drought or disease incidence. However, the environments must be sufficiently similar for one genotype to perform well in all of them.

How to decide on the number of TPE for your breeding program?

Your breeding program must define the TPE for which you plan to provide an improved variety. In rainfed environments, genotype × environment interactions (GEI), or the tendency for genotypes to rank differently in different environments, may be large. Under these circumstances, several TPE, each served by different varieties, may be optimal. This is very different from irrigated rice, where the TPE can be very large, as in the example of IR36 grown on 11 million hectares!

However, since each new TPE served will need additional breeding and testing resources (see Section 3.1), there will be a practical limit to the number of TPE served by a breeding program. In some TPE, the size of the target area will be inadequate to justify the resources required for a separate effort, and breeders must rely on the “spillover” of a variety from another TPE.

Sec. 3.1

To define the target environment, you need to know the timing, severity, and frequency of drought.

Drought makes it difficult to define the TPE

In rainfed agriculture, the variation in available moisture for crop growth is a major determinant of the TPE. This variation has a predictable component depending on average rainfall (climate), position in the toposequence, and soil type. It also has an unpredictable component depending on rainfall patterns (weather), which determine the availability of water at different crop stages, and on the farming system, which determines the planting time and therefore the development of the crop.

Information is usually adequate about the predictable variation in water supply at least at the regional/district level. There is less information on the spatial distribution at the field-toposequence level. However, the most difficult aspect is to estimate the unpredictable environment caused by drought. We need a breeding strategy that maximizes returns in good years (i.e., breeding for high yield potential) and provides food in poor years (i.e., breeding for drought tolerance). Thus, in defining the TPE, we must also determine the probability of occurrence of drought over time.

How do we determine the TPE for rainfed lowland rice?

Four interdependent approaches are used to analyze the environment, ranging from a wide spatial characterization (subecosystems) to defining drought "types" that occur in some farmers' fields in some years.

1. Start with the spatial information on water availability at the subecosystem level

A commonly used system for characterizing rainfed lowland systems is that of subecosystems defined by Khush (1984) and later modified by Mackill et al (1996). Three of these subecosystems are relevant to breeding for drought tolerance:

- Rainfed, shallow, favorable subecosystem, where rainfall and water control are generally adequate for crop growth, and only short periods of drought stress or mild submergence occur.
- Rainfed, shallow, drought-prone subecosystem, with either a short rainy season or a long and bimodal rainy period.
- Rainfed, shallow, drought- and submergence-prone subecosystem, where drought and submergence may occur within the same growing season or in different seasons.

Singh et al (2000) provide an overview on many studies using the ecosystem approach and Figure 1 gives an example of characterizing the rainfed lowland rice subecosystem in eastern India. This level of characterization is useful for a national system to acquire new germplasm from other breeding programs that is adapted to the same subecosystem. However, it is not adequate to define a TPE for the development and delivery of new varieties nationally. Further classification and definition are necessary.

2. Use the knowledge and experience of farmers and breeders to characterize local environments

Farmers, agronomists, and breeders who are familiar with a field and have observed rice crops grown in it over several years can usually quickly and accurately determine the type of drought risk it is subject to. This is largely a function of toposequence position (Fig. 2) and soil texture. Upper terraces, particularly those with light soils, are most subject to drought risk. Using the

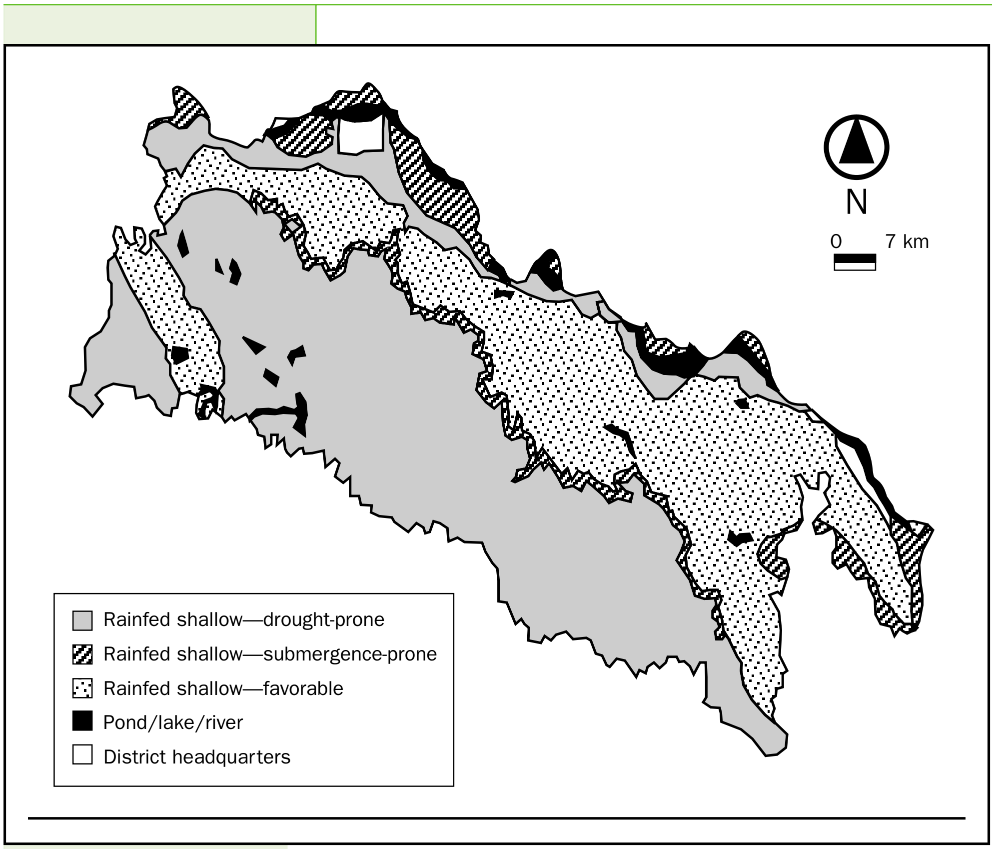
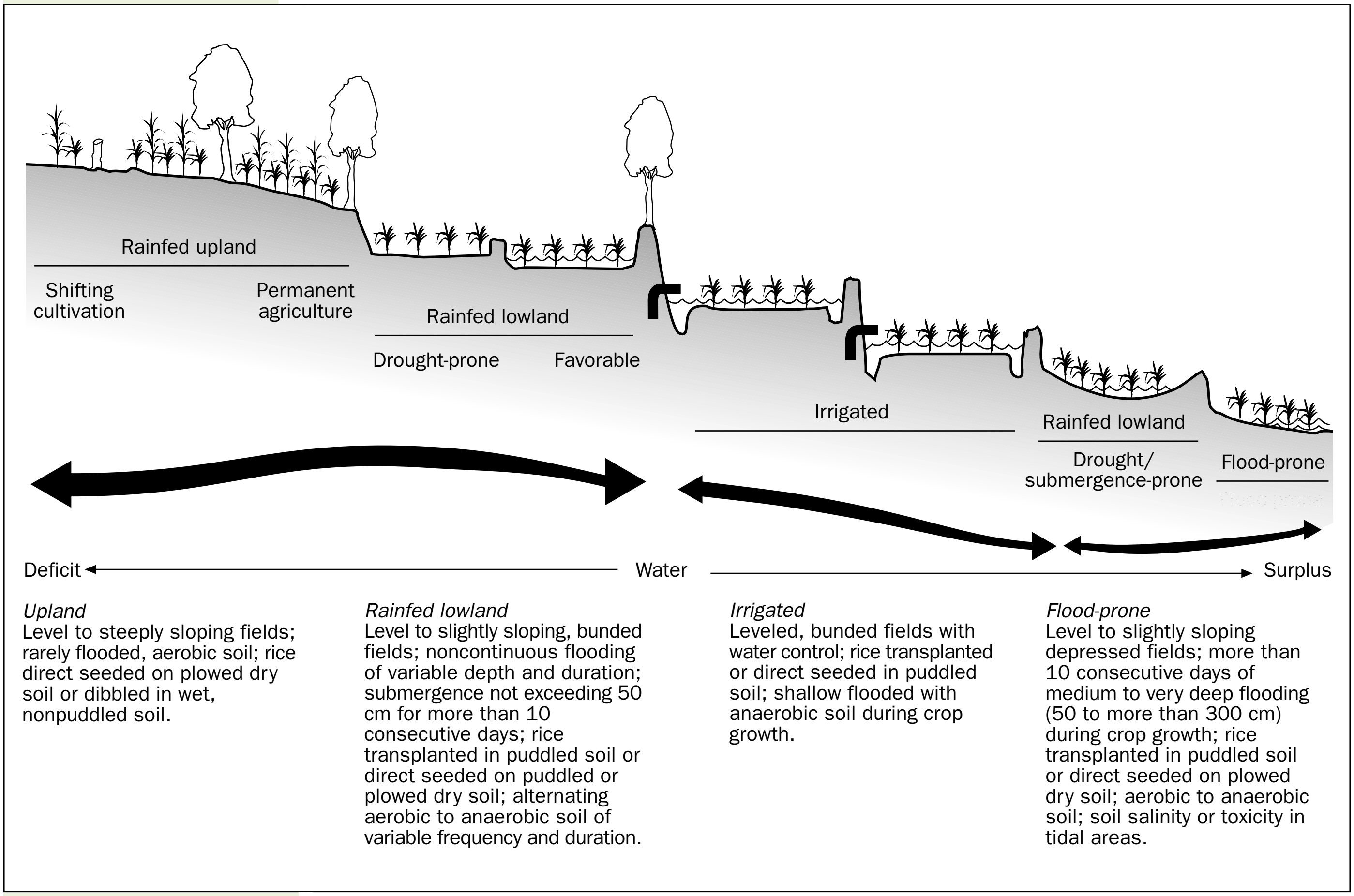


Fig. 1. Characterization by subecosystem to define target environments in Faizabad District of Uttar Pradesh, India (Singh and Singh 1996).



Upland
Level to steeply sloping fields; rarely flooded, aerobic soil; rice direct seeded on plowed dry soil or dibbled in wet, nonpuddled soil.

Rainfed lowland
Level to slightly sloping, bunded fields; noncontinuous flooding of variable depth and duration; submergence not exceeding 50 cm for more than 10 consecutive days; rice transplanted in puddled soil or direct seeded on puddled or plowed dry soil; alternating aerobic to anaerobic soil of variable frequency and duration.

Irrigated
Leveled, bunded fields with water control; rice transplanted or direct seeded in puddled soil; shallow flooded with anaerobic soil during crop growth.

Flood-prone
Level to slightly sloping depressed fields; more than 10 consecutive days of medium to very deep flooding (50 to more than 300 cm) during crop growth; rice transplanted in puddled soil or direct seeded on plowed dry soil; aerobic to anaerobic soil; soil salinity or toxicity in tidal areas.

Fig. 2. Characterstics of rice ecosystems.

knowledge of experienced farmers and researchers is the most accurate and simplest approach for assigning fields to a particular TPE. As a general rule, drought risk is most severe in entirely rainfed upper fields in which standing water rarely accumulates, and in which farmers grow short-duration, photoperiod-insensitive varieties.

3. Use the performance of known varieties to define the TPE

IRRISTAT

Most breeding programs routinely collect data from variety trials grown over all environments, called multienvironment trials (METs). You can reanalyze these historical data using the statistical package IRRISTAT to determine the "clustering" or grouping of environments based on the correlation of variety means across trials.

The results can be used to define the TPE. Rajatasereekul et al (1997) used this approach to define three broad domains for the rainfed systems of Thailand and Lao PDR and, from that, the duration of preferred varieties.

There is a simple way to group locations and fields into the TPE, using the correlation of variety means from trials testing the same set of varieties. The repeatability (also known as the broad-sense heritability, or *H*) of a 3- or 4-replicate trial usually ranges from 0.3 to 0.4. This is also the expected correlation of variety means in trials conducted in different fields if there is not much GEI between them. Thus, if the correlation between cultivar means in trials conducted at two different sites is consistently 0.3 or greater, they can be safely included in the same TPE. This method of grouping environments in the TPE should be used only if data from trials containing 20 or more varieties are available over several years.

Be cautious in using this approach. First, make sure that the trials/locations are representative of the TPE (i.e., the farmers' fields) and that crops are not grown only at the experiment station (and often with water). Second, do not exclude trials that did poorly because of drought.

Our experience from several analyses of METs shows that there is a large nonpredictable component of GEI (associated with year-to-year variation) as well as a large error component. This makes it difficult to define consistent patterns for the grouping on the basis of locations (Cooper et al 1999) and requires large data sets to estimate frequencies of environmental types based largely on variable water conditions. Since our aim is to develop varieties with adaptation to these water conditions, we need to know more about the patterns of water supply and the types of drought. The GEI analysis needs to be supplemented with measurements of the water supply at the local level. (See next section.)

The process of defining the TPE is an ongoing one. Since most breeding programs conduct METs, a few modifications can improve the data for the continuing process of the TPE definition.

- Select "probe" varieties with contrasting differences in important traits (i.e., early or late, photosensitive or insensitive) as reference lines.
- Test these varieties under representative conditions, including farmers' fields.
- Measure the water environment of the MET (see next section).

Box 1. **The factors that determine the water supply (S) for crop growth are**

$$S = R - (E + T + P + L + O)$$

where R = rainfall, E = evaporation (mainly determined by leaf area and moisture content of the soil surface), T = transpiration (mainly determined by leaf area and water availability in the root zone), P = percolation and drainage (mainly determined by soil type), L = lateral flow of water (mainly determined by position on the toposequence), and O = runoff of water above the bund. In rainfed lowland rice, R, P, and L largely determine S. Of these, L can be up to 50% of the rainfall that occurs at occurs at a time when there is standing water in the paddy. Thus, the position in the toposequence will have a large effect on the depth and duration of available water from a given rainfall event.

4. Measure the water supply at the field and plot level

You can monitor water supply (S) (see Box 1) during crop growth to determine the timing and severity of drought to further define the TPE.

It is important to measure S in all trials as shown in Section 3.2.2

The pattern of water level recorded over the season can be used to characterize three different types of drought, as shown in Figure 3:

Sec.3.2.2

- an early drought that occurs during vegetative growth,
- an intermittent midseason drought that occurs between tillering and mid-grain filling, and
- a late drought that occurs during flowering and grain filling.

These are the main types of drought found in the rainfed lowlands (Chang et al 1979, Fukai and Cooper 1995). In addition to knowing the frequency, it is also important to know the severity. For this, you will need to compare the yields under the drought and irrigated conditions, or , if you cannot irrigate, choose a well-watered site such as the bottom of the toposequence.

The frequency and severity of drought, and the frequency of favorable water supply, are used to further define the TPE for the breeding program.

The objective is to use the four approaches—subecosystem, farmer knowledge, GEI, and drought type—to define the TPE and the breeding strategy for rainfed lowland rice. When the drought type is associated with particular locations, it effectively defines the TPE. The breeding strategy can then be developed for that TPE based on specific adaptation to the prevalent water supply:

- yield potential for favorable conditions,
- drought escape (early maturing) for terminal stress, and
- drought tolerance for all stress conditions, but particularly intermittent stress.

However, when large year-to-year variation occurs in the type of drought, no one drought type can define the TPE. Under these conditions, breeders need to balance selection criteria to reflect the likelihood of each drought type in the TPE. For example, there may be a 50% frequency of favorable water supply and 50% frequency of intermittent drought, thus requiring a balance in selection for yield potential and drought tolerance. The important thing is to know which drought type occurred in each nursery and make sure that material that is well adapted to other frequently occurring drought

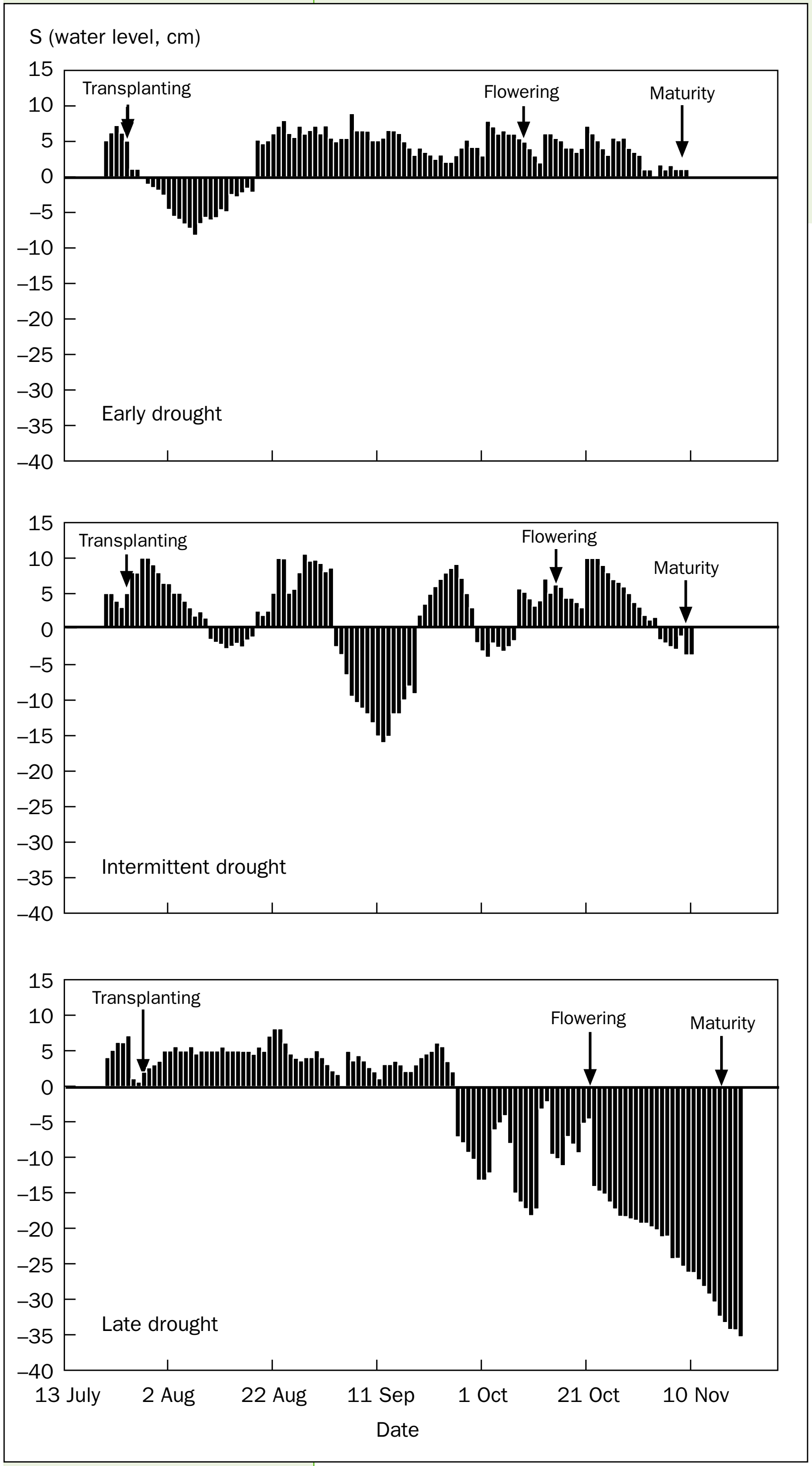


Fig. 3. Three examples of the seasonal pattern of the level of free water, above and below ground level, measured in the field and used to describe different types of drought.

types is retained among the selected lines. Otherwise, a cyclic pattern of genotypes adapted to different drought types can limit progress in selection.

How do we determine the TPE for the upland rainfed system?

1. Start with rainfall patterns

In upland rice, water availability for crop growth depends largely on rainfall patterns rather than on total rainfall and land and soil properties that influence infiltration. The upland system is generally poorly buffered against variation in rainfall because it cannot store as much water as the lowland system. Short periods without rainfall (around 7 days) are most damaging if they occur just after sowing, when roots are poorly developed. Periods without rain can also cause spikelet sterility during the critical period from about 10 d before anthesis to 5 d after anthesis. As a general guideline for tropical areas,

- Flowering-stage stress will generally be significant after 7 days without significant (>5 mm) rainfall.
- For each additional day without rainfall during this critical stage, yield will decrease by about 10%.
- Courtois and Lafitte (1999) have used this approach for a regional characterization of the uplands (Table 1).

Record the water supply as a routine in all trials.

2. Measure the water supply at the plot/field level

The water supply during crop growth can be estimated using a simple water balance model based on weather data and knowledge of soil texture and depth at a site.

Starting from a soil at field capacity, use the following as a guide to make your estimate of water use:

- Water content at field capacity can range from about 10 mm (sandy soil) to 20 mm (heavy soil) per 10 cm of soil.
- Rice grows well until about 30% of the available water is extracted. This means that the crop will have 3 to 6 mm of water available per 10 cm of rooting depth.
- Rice roots of many indica varieties below 60 cm seem mostly ineffective in water uptake, so their maximum rooting depth is probably 60 cm.

Table 1. Characteristics of the three major agroecological zones of upland rice production in Asia leading to the definition of different types of water environments.

Agroecological zone	Aus (short rainy season)	Hilly subhumid	Equatorial humid
Location	Eastern India (Assam, Bihar, Orissa, Madhya Pradesh, Uttar Pradesh, West Bengal), Bangladesh	Northeast India, northern Myanmar, Lao PDR, North Vietnam, northern Thailand, South China (Yunnan), Indonesia	Indonesia (Sumatra, Kalimantan), Malaysia, South Vietnam (Hauts Plateaux), southern Philippines (Mindanao)
Latitude	30° to 20°N	30° to 15°N	15°N to 5°S
Longitude	80° to 95°E	90° to 110°E	95° to 125°E
Elevation (m)	100–150	300–2,000	300–1,000
Rainfall (mm)	800–1,400	1,200–3,000	>2,500
Length of rainy season	3 mo	4–5 mo	>5 mo
Rainfall pattern	Monomodal	Trend to bimodality	Monomodal
Risk of drought	High	Moderate	Moderate to low
Type of drought	Terminal + intermittent	Intermittent	Intermittent
Drought intensity (wk)	4–5	2–3	1–2

Source: Courtois and Lafitte (1999).

- In soils with high acidity, plow pans, or other conditions that encourage surface rooting, rooting depth will be much less. You therefore need to measure the depth of effective rooting for your site.
- If the roots extend to 60 cm, the crop can extract from 18 to 36 mm of water—enough for 6 to 11 d of transpiration in the humid tropics during the vegetative and grain-filling stages or 4 to 7 d of transpiration during the critical flowering stage. If the rooting depth is only 30 cm, a crop starting at field capacity can grow for only half this long before it begins to experience water stress.

Use the rainfall and estimate of water use to develop a simple water balance for your crop to define the frequency and type of drought.

There is a trade-off between precisely defining the TPE and achieving enough replication within it

It is not feasible to develop varieties for very small target environments.

Even when the TPE has been precisely defined, there will be random rank changes in variety means from site to site and from year to year that cannot be explained by differences in water status. This is because many factors, such as pest damage, disease, and measurement error, routinely affect yield data collected in field trials. These "noise" factors are known to be very large in rainfed lowland rice, and they can be overcome only through adequate replication within and across environments. If the TPE served by a breeding program is too narrowly defined, budget considerations will allow only one or a few trials to be conducted within each TPE. When genotype means are estimated from only one or two trials, least significant difference (LSD) values are very large, preventing accurate evaluations from being made and reducing progress from selection. In general, the TPE must be large enough to support three to five testing sites. This problem is explained in detail in Atlin (2001).

Notes

Authors' addresses: K.S. Fischer and S. Fukai, University of Queensland, Brisbane, Australia; R. Lafitte and G. McLaren, International Rice Research Institute, DAPO Box 7777, Metro Manila, Philippines.

SECTION

3

Breeding to improve yield under adverse environments: direct selection for grain yield

Section 3.1 sets out some general guidelines based on theory and experience for breeding for stress environments with a focus on drought. The approach considers breeding drought-tolerant rice by direct selection for grain yield. (In Section 4, we describe how to use additional traits—indirect selection—to improve drought tolerance.)

Because natural drought is unpredictable, we discuss how a "managed" drought nursery can increase the efficiency of selection (Section 3.2). We also describe field techniques and statistical analyses that reduce error variance and increase the precision of selection (Section 3.3). And, in Section 3.4, we provide information on the choice of parents and on the importance of on-farm testing in the selection process.

SECTION 3.1

Improving drought tolerance by selecting for yield

G. Atlin

Background theory and some terms and definitions

Breeders create new gene combinations and useful variability among genotypes by intercrossing parents that possess desirable characteristics or by introducing new germplasm from another breeding program. Breeders use a step-wise selection procedure to screen the best-performing genotypes in early generations, given limited resources. First, many genotypes are evaluated with few or no replicates at a few sites in pedigree nurseries in which adapted lines with the required quality traits, maturity, disease resistances, and plant type are selected. Next, these selected lines are screened in replicated yield trials, usually on-station. Some of these trials may be conducted in *managed-stress environments*, or screening environments that have been designed to predict performance under natural stress. Next, the more successful genotypes or their descendants are evaluated with more replicates and at more sites (multiple-environment trials, or METs) (Bänziger et al 2000). Finally, concurrent with advanced MET testing, promising varieties should be evaluated in farmers' fields, under their own management.

Most selection is done in selection environments (SE) that are designed to be representative of the target population of environments (TPE). The SE is the breeding nursery, screening trial, or MET in which potential varieties are evaluated. The SE is useful only if it reliably predicts grain yield in the TPE. Thus, the objective of evaluation in the SE is not to measure performance in that nursery or trial, but to predict performance in the TPE. Trials and nurseries are not themselves the TPE; at best, they sample it in a representative way. At worst, they are unrepresentative of the TPE and do not predict performance in the TPE. Understanding this purpose is critical to the design of the screening protocols at the research station.

Drought-tolerant varieties produce more grain under stress.

This section considers the factors that govern response to selection in the SE and expression of that response in the TPE. In the discussion that follows, we use a simple definition for drought tolerance in terms of yield.

A drought-tolerant variety is one that produces a high grain yield relative to other cultivars under drought stress.

This definition helps clarify the main objective of a drought-tolerance breeding program: to breed varieties that outyield currently available varieties in the TPE under the types of drought stress that occur most frequently. If drought occurs only in some years (see Section 2), cultivars produced by drought-tolerance breeding programs should also produce high yields in the absence of stress. All decisions about how to breed for drought tolerance should be evaluated in this light. If a drought-screening method or SE cannot reliably identify varieties with improved grain yield under stress in the TPE, it should not be used.

Sec. 2

According to Falconer (1952), breeders make the most selection progress in the TPE when

- Differences (i.e., genetic variance) among genotypes are large.
- Selection intensity is high, that is, many genotypes are screened and only a few are selected. (The selection intensity, or the proportion of the population that is retained after screening, is a critical component of selection response. Selection intensity is expressed as the standardized selection differential [i_S], or the mean of the selected group expressed as a deviation from the population mean and divided by the phenotypic standard deviation.)
- Broad-sense heritability is high, that is, traits that are valuable in the TPE can be assessed precisely in the genotypes evaluated.
- The genetic correlation (r_G) between yield in the selection environment and the TPE is high. This correlation is a measure of the degree to which yields in the TPE and SE are controlled by the same genes.

Repeatability or broad-sense heritability (*H*) is the proportion of the variance among line means that is explained by genotypic differences. It is a measure of the reliability or precision with which you can detect differences under a given selection protocol. Another way to think of *H* is as the expected correlation between variety means estimated in different sets of trials in the same TPE. If *H* is high, the means of a set of cultivars tested in different trials will be highly correlated. If *H* is low, there will be little association between means from different trials. *H* is a critical component of selection response. If *H* is low, progress from selection will be low. *H* is subject to manipulation through the design of a screening program. The factors affecting *H* are easily recognized through inspection of the formula for the heritability of line means in a MET (shown in Box 1).

Box 1. **Broad-sense heritability (*H*) of line means in a multi-environment trial (MET).**

$$H = \frac{\sigma^2_G}{\sigma^2_G + \frac{\sigma^2_{GL}}{l} + \frac{\sigma^2_{GY}}{y} + \frac{\sigma^2_{GLY}}{ly} + \frac{\sigma^2_E}{rly}}$$

where σ^2_G, σ^2_{GL}, σ^2_{GY}, σ^2_{GLY}, σ^2_E, l, y, and r are the genotype (G), genotype × location (GL), genotype × year (GY), genotype × location × year (GLY), and within-trial error variances and the number of locations, years, and replicates of testing, respectively. σ^2_G, σ^2_{GL}, σ^2_{GY}, σ^2_{GLY}, and σ^2_E are estimated from METs repeated over locations and years within the TPE.

It is important for breeding and cultivar testing programs to estimate these parameters, which can be calculated from MET data using standard statistical software packages such as SAS and GENSTAT, even for data sets that are not balanced over locations and years.

How to increase response to direct selection for yield

1. Ensure that the SE is representative of the TPE

Breeders select lines in the SE but their efforts are only successful if yield gains are expressed in farmers' fields (i.e., in the TPE). Performance in the TPE and the SE can be thought of as correlated traits expressed by a single genotype in separate environments. This relationship is measured as the **genetic correlation (r_G)**. Thus, the r_G is an indicator of the accuracy with which performance in the target environment (TPE) can be predicted in the selection environment (SE). An r_G value of 0 indicates that there is no association between performance in the selection and target environments. An r_G value of 1 indicates that the SE is perfectly predictive of performance in the TPE.

Selection is effective when testing environments are similar to the target environment.

Breeders should be aware that there is a "chain of correlation" between performance in a screening environment and performance in farmers' fields. There are several important links in this chain. For example, consider the breeder who wishes to use a rapid screenhouse test of drought tolerance, under the assumption that drought tolerance in pots in a screenhouse is predictive of drought tolerance in farmers' fields in the TPE. The "chain of correlation" that might describe this assumption is

$$\begin{tikzcd}\text{Screenhouse} \arrow[r, "r_{G1}"] & \text{Research station field} \arrow[r, "r_{G2}"] & \text{Farmer's field}\end{tikzcd}$$

Before embarking on a screenhouse-based screening program, the breeder therefore needs to test the assumption that performance in the screenhouse is predictive of performance in the research station field (r_{G1}) and that performance in the research station field is predictive of performance on-farm under farmer management (r_{G2}). Methods for testing these assumptions appear in Box 2.

Box 2. **Calculating the genetic correlation (r_G) between the SE and TPE.**

Before investing time and money in a managed-stress screening technique, breeders should make an effort to find out if performance in the screen (the SE) really predicts performance in farmers' fields in the TPE. This is done by screening a diverse set of lines both in the SE and in the TPE, and estimating r_G between variety means in the two sets of environments. There are several methods for calculating r_G between a selection and target environment. Two are listed below:

1. ***The phenotypic correlation (r_P) can be used as an approximation of r_G***. The phenotypic correlation is simply the correlation between variety means estimated in the SE and means of the same lines estimated in the TPE. It usually underestimates r_G because lack of correlation between sets of variety means in two different trials or environments has two causes:

- *True G×E interaction (the differing adaptation of varieties to the two environments).*
- *Experimental error, or lack of repeatability of the results of cultivar trials.* Even the best-conducted field trials have a large amount of random plot-to-plot variability. This variability reduces the correlation between the results of different trials involving the same set of varieties, even if they are conducted on the same soil type, under identical management, and in the same season. The expected value of the correlation between two independent trials of a single set of varieties conducted in the same TPE is H (see Box 1); therefore, as the number of trials and replicates from which variety means are estimated increases, r_P becomes an increasingly good esimator of r_G. As a rough guide, if r_P between means from one trial in the SE and one in the TPE is 0.6 or higher, r_G is likely to be close to 1. If variety means are estimated in the SE and TPE, each from three or four trials with two or three replicates, then r_P is likely to be a good estimate of r_G.

Box 1

2. ***r_G can be estimated as r_P "corrected" for the low repeatability of means estimated from field trials***, as follows:

$$r_G = r_P / (H_{SE} H_{TPE})^{0.5}$$

where H_{SE} and H_{TPE} are estimates of broad-sense heritability or repeatability from the selection environment (see Box 1 in this section). *Note that r_P, H_{SE}, and H_{TPE} must all be estimated from the same set of trials for this method to be valid.* This estimation method corrects the correlation (between variety means in the selection and target environments) for the fact that variety means are always estimated with error. Inspection of this formula shows that, as the number of trials and replicates used to estimate variety means increases, r_P becomes an increasingly good estimator of r_G. It should be noted that this estimator of r_G, like most others, will sometimes result in values greater than 1 or less than –1, although the true range of a correlation coefficient lies within these limits. Anomalous values result from the fact that estimates of H have very large standard errors. If H is underestimated when the real value of r_G is close to 1, the estimate provided by this method may exceed 1. Estimates greater than 1 can be taken to mean that r_G is close to or equal to 1.

In practical terms, how can we estimate r_G between the SE and TPE for a drought-screening method?

a. Assemble a set of at least 30 varieties (preferably 40 or 50) that exhibit a range in drought tolerance.
b. Evaluate the varieties in a set of two or three trials in the SE and two or three trials in the TPE.
c. Calculate the variety means in the SE and TPE, and then calculate their correlation (r_P).
d. Do separate analyses of variance on the data from the SE and TPE trials to estimate variance components for use in estimating H_{SE} (from the SE variance components) and H_{TPE} (from the TPE variance components) as in Box 1. (Methods for estimating variance components are beyond the scope of this manual; consult a statistician or refer to a textbook on statistics or biometrics, such as Kempton and Fox (1997) and Hill et al (1998).
e. Use the estimates of r_P, H_{SE}, and H_{TPE} to calculate r_G with the equation above.

To maximize r_G between the SE and the TPE:

- Ensure that conditions at the research station (nursery and trials) are similar to those in farmers' fields. (Note: Selection is often conducted at research stations under management regimes that are not representative of those used by farmers. This type of selection may be justified in terms of selecting for yield potential or maximizing the precision of yield trials, but breeders must ensure that performance on-station is predictive of performance on the farm.)
- Use two kinds of screening trials—one that predicts performance in drought years and one that predicts performance in favorable years. For the design of the managed-drought screening trial, see Section 3.2. (Note: Nurseries in which managed levels of stress are purposefully applied are useful in ensuring that r_G is maximized for stresses, such as drought, that occur sporadically in the TPE. It is important to verify that the results of managed-stress trials really are predictive of performance on-farm.)

Sec. 3.2

- Select directly in the target environment, that is, on-farm. For on-farm screening, the correlation between performance in the selection and target environment is necessarily 1, assuming that representative farmer-cooperators have been chosen. On-farm screening should therefore be a component of all breeding programs in which any uncertainty exists about the predictive power of on-station screening. (Note: On-farm trials can be expensive and imprecise because of variability caused by weeds and low fertility, and subject to a high risk of failure. On-farm testing programs must therefore be carefully designed and conducted to avoid wasting money and time, and to maximize the reliability of the data obtained. Use the robust experimental designs discussed in Section 3.3.)

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- Irrigate only if your objective is to measure yield potential.
- Use data from trials affected by drought *even when* the CV is large. (Note: The inherent variability of stressful environments is often high, Atlin and Frey 1989.) This has important implications for the use of data from METs and on-farm trials in selecting drought-tolerant materials. Often, trials with high coefficients of variation (CV) are omitted from the analysis. However, these are often the trials in which stress was most severe. Omitting high-CV trials almost always introduces bias into the sampling of the TPE in favor of more favorable environments. You can avoid this bias by not using an arbitrary CV value as a criterion for accepting or rejecting a certain on-farm or off-station trial. If no obvious errors have been made in layout or data collection, results from low-yield, high-CV trials should be retained; these are often precisely the trials that are the most informative about cultivar performance in stressful environments.
- Select genotypes that perform well under both drought and well-watered conditions. Varieties that perform well in both types of SE can usually be identified because r_G across drought stress levels is usually positive in other crops (e.g., Atlin and Frey 1989, Bänziger et al 1997) and there is evidence that r_G is also usually positive (sometimes with a low value) in rice grown under a range of water-stress environments (Lafitte and Courtois 2002 and G. Pantuwan, personal communication).

Large numbers of lines in early generation testing allow you to increase selection intensity.

2. Increase the selection intensity

Drought-tolerance breeding programs must be large to make progress. In most rainfed rice breeding programs, only a few lines (usually fewer than 50) are tested in the replicated MET at several locations, although this is the selection phase most responsible for making gains in stress environments (Cooper et al 1999b, Becker 1984). Although increasing selection intensity is expensive, it is a simple and sure way to increase selection response! Initial populations of lines evaluated must be large enough to permit intensive phenotypic selection for highly heritable quality, plant type, and pest-resistance traits, while retaining a population with adequate genetic variation for progress to be made for yield and drought tolerance. If little selection pressure for yield under drought stress is applied, little progress will be made.

Reducing the proportion selected from 20% to 10% will increase predicted response by approximately 20% in a population of 100 lines. But, to achieve this increase in selection intensity for drought tolerance, while retaining enough lines to ensure adequate selection pressure for disease resistance, quality, and other important traits, the total number of lines screened must be doubled.

For a small rainfed rice breeding program focusing on drought tolerance and producing 1,000 new F_6 or F_7 lines per year from its pedigree breeding program, an appropriate distribution of effort might look something like the scheme below:

Preliminary managed-stress screening:
N = 1,000

Preliminary replicated yield testing under stress
N = 200

METs—advanced lines
N = 100

Participatory on-farm testing
N = 20

The following techniques can increase the number of plots and therefore the number of entries (with the same resources):

- Use augmented experimental designs (see Section 3.3) that maximize the number of entries for given resources.
- Use micro-plots and visual rating scales judiciously (see next section).
- Use screening methods that are inexpensive and able to handle large numbers.

Sec. 3.3

3. Increase heritability

A. Broad-sense heritability *(H)* must be maximized through careful management of drought-screening nurseries and by high levels of replication within trials and across sites and years.

Box 1

The equation in Box 1 (see page 15) shows that there are several ways to increase *H*:

- Increasing the number of replicates per trial
- Increasing the number of trial locations
- Increasing the number of years of testing

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It is important to reduce the error (σ^2_E) variance to detect real differences between lines. In our experience, the genotype × location × year (σ^2_{GLY}) and the error (σ^2_E) variance are the largest contributors to the random noise in field trials. The contribution of σ^2_E can be reduced by choosing uniform test sites, increasing within-site replication, adopting improved methods of controlling within-block error (for example, lattice designs or neighbor analysis), or increasing the number of locations or years of testing. We recommend using the experimental designs described in Section 3.3.

Use the best experimental designs to control field variation.

The contribution of σ^2_{GLY} can be reduced only by increasing the number of tests across locations or years. This is expensive and must involve

- Cooperation among research centers in collaborative networks for the early stages of yield testing, rather than extensive testing at a single center until advanced stages (Cooper et al 1999b).
- Increasing the number of test locations rather than the number of replications at each site.

Note 1: In rainfed rice METs, both within-site residual variance and G × L × Y variance tend to be large and much more important than G × L or G × Y variance (see Box 3). Increasing either the number of trials or the number of replications per trial will usually increase selection response, but increasing the number of trials will have the greater effect.

Note 2: The relative effects of increasing the number of sites, years, and replicates of testing on *H* can be estimated as shown in Box 3. Clearly in this example, there is little benefit from increasing the replicate number from 2 to 4 in a single trial, whereas there is a large increase in *H* from increasing the number of trials from 1 to 5.

Increasing the number of locations is more effective than increasing the number of replicates per location.

Increasing the number of replicates (without increasing the number of trials) is less expensive but also less effective in increasing heritability!

Box 3. **An example of estimating the relative effects of increasing replications, sites, and years on heritability (*H*) and some estimates of variance components for the rainfed lowlands and uplands.**

To estimate the effects of sites, years, and replications:

- Use the equation for *H* as described in Box 1.
- Use the estimates of variance for genotype (σ^2_G), genotype × location (σ^2_{GL}), genotype × year (σ^2_{GY}), genotype × location × year (σ^2_{GLY}), and within-site residual (σ^2_E) variance components for yield. The following values are estimated from rainfed lowland rice trials conducted at six sites in northern and northeast Thailand (1995-97) and high- and low-yielding upland rice trials conducted at three Philippine sites (1994-96):

Ecosystem	Region	σ^2_G	σ^2_{GL}	σ^2_{GY}	σ^2_{GLY}	σ^2_E
Rainfed lowland rice[a]	Thailand	198	82	18	199	178
Rainfed lowland rice[b]	Thailand	60	3	49	259	440
Upland rice: low-yield trials[c]	Philippines	5	0	0	63	27
Upland rice: high-yield trials[c]	Philippines	12	9	0	34	39

[a]Cooper and Somrith (1997): selected set of advanced lines. [b]Cooper et al (1999b): random sample of breeding lines. [c]Atlin (unpublished data). *In the example of Cooper and Somrith (1997) for the Thai rainfed lowland cultivar data, increasing the number of sites from 1 to 5 increased *H* from 0.39 to 0.74, increasing the number of years of testing from 1 to 2 increased *H* from 0.55 to 0.71, and increasing the number of replications per trial from 2 to 4 changed *H* only from 0.64 to 0.67 (data not shown).

Sec. 2

B. Increasing heritability by exploiting genotype × environment interactions

In some circumstances, GEI variance is not noise but evidence of specific adaptation of particular cultivar types to particular environments (as discussed in Section 2). When regions or land situations differ enough to cause rank changes in cultivar performance, dividing the target region may be warranted (Atlin et al 2000, and Section 2).

For example, some of the variation in rainfed lowland rice cultivar performance across trials in northeast Thailand was associated with different responses of cultivars to variation in the time of occurrence of drought. This GEI results from the fact that short-duration cultivars avoid terminal drought stress and therefore outperform later cultivars when the onset of drought is relatively late (Cooper et al 1999a). If the drought type is reliably associated with particular locations or toposequence positions within the target region, then dividing the TPE into two subregions for breeding purposes may be warranted, thus permitting the development of different cultivars for each subregion. However, Atlin et al (2000) have pointed out that subdivision of the TPE also usually results in a subdivision of testing resources, thereby reducing H because of fewer test sites within each subregion. Gains from the exploitation of local adaptation must more than outweigh the disadvantage of reductions in H for subdivision to be warranted.

4. Some suggested changes to conventional breeding programs

A. Choice of parental material

Choosing parents is one of the most important steps in a breeding program. No selection method can extract good cultivars if the parents used in the program are not suitable. Although breeders have different approaches to parent choice (see Sections 5.1 and 5.3) and have achieved success in different ways, many successful crosses have some common features:

- Use at least one locally adapted, popular cultivar as a parent. This helps ensure the recovery of a high proportion of progenies with adaptation and quality that are acceptable to farmers. If quality requirements are very important and if the local variety is highly preferred by farmers, a backcross to the local variety may be required to reach an acceptable level of quality.
- Choose each parent to complement the weaknesses of the other. For example, if both parents are susceptible to an important disease, it is highly unlikely that many offspring will be resistant. When breeding for drought tolerance, avoid parents that are highly drought-susceptible. Sections 5.1 and 5.3 describe how to select progenitors that have traits conferring drought tolerance.
- Use improved modern varieties in crosses with an adapted parent. Often, elite modern varieties have many disease-, insect-, and abiotic stress-tolerance genes that local ones lack.

Sec. 5.1
Sec. 5.3

B. Summary of recommendations for early screening and MET testing programs

- Include as many lines as possible for yield evaluation in the SE, as early as possible in the breeding process. Most breeding programs select strongly for characters other than yield in the early generations of breeding and do yield testing only at the late stages, when most material has already been discarded. While it is very important to select lines with appropriate maturity, quality, and disease reaction, it is also important to retain a large sample of lines that can be selected for the most critical trait!

- Use a managed-drought screening trial that is representative of the TPE.
- Increase the number of tests (lines and sites) with the same resources. Although increasing the number of tests is a sure way of increasing selection response, it can be expensive. If plot sizes are not reduced or operations streamlined, increased replication will result in proportional increases in the land, labor, and other inputs in a field trial or nursery. Most breeding programs operate on strictly limited resources and cannot easily increase the number of plots they handle without changing the way plots are managed.
- Evaluate in your own breeding program the optimum balance among plot size, replication, and precision!
- Reduce the cost per plot, particularly in the early generations, by
  - Using micro-plots. Very small plots, often referred to in the plant-breeding literature as *micro-plots*, can be used for preliminary yield evaluation. Micro-plots are short single rows or hills. Breeding programs can manage far more micro-plots than conventional-sized plots, thus permitting replicated yield evaluation of large breeding populations. (Note: Micro-plots are subject to competition effects [taller genotypes tend to be favored in small plots] and usually have higher error variances than larger plots, and therefore estimate yield with less precision. Thus, increases in selection response resulting from increased replication and selection intensity achievable through small plots may be partly lost through the reduced precision of small-plot trials. *A large sample of lines must be retained after micro-plot screening for evaluation under more competitive conditions.*)
  - Using visual estimates of grain yield in replicated trials to discard low-yielding lines. Under conditions of severe stress, visual ratings of seed set and grain yield by experienced workers can be nearly as effective and repeatable as yield measurements, particularly when there is a lot of variation among lines in the nursery or trial. (Note: Genetic correlations of 0.9 between visual yield ratings and measured grain yields were observed in a drought-stressed upland trial at Siniloan, near IRRI, in the wet season of 2002.) About one-half of the breeding lines in a replicated yield trial conducted under moisture stress can be safely discarded on the basis of a visual yield score. A simple 1-to-5 or 1-to-10 rating system can be used for this purpose. After preharvest elimination of unproductive lines on the basis of the visual score, the remaining lines are harvested and selection is done on the basis of measured yield and other important traits.
  - Eliminating poor-performing lines in a trial before harvest. This greatly reduces harvest and seed-processing labor, but requires that the breeding program have the capacity to analyze the visual rating scores quickly to make selections before the crop is completely mature.
- Establish a network of METs for maximum testing at many sites instead of intensive testing at one site.

C. All breeding programs should include participatory on-farm trials

To ensure that selection has been effective, and that progress made at the station will be transferable to the farm, on-farm trials, managed by farmers, should be part of the testing of a new cultivar.

- Include as many cultivars as possible in participatory testing by farmers in their fields.
- Consider the use of mother-baby (see Bänziger et al 1997) trials to maximize the number of genotypes tested.
- Run participatory trials concurrently with advanced METs.

- Testing for grain quality, in consultation with farmers from the TPE, is cheaper than replicated yield testing. Hence, quality screening should be done before METs to discard varieties with quality unacceptable to farmers.

Additional reading

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Notes

Author's address: International Rice Research Institute, DAPO Box 7777, Metro Manila, Philippines.

SECTION
3.2

Managing water for controlled drought in breeding plots

R. Lafitte

How can you manage your drought environment?

When drought occurs, plot-to-plot variability becomes more of a problem.

1. Start with a uniform field and apply all inputs uniformly

When fields are well irrigated, they often appear uniform. As drought develops, however, differences in topography, slope, soil texture, and field history can have a large effect on plant growth. Choose a level field with minimum variation in soil depth or texture. Not all the variation in a field can be seen from the surface—observations of weed or crop growth in a previous season can give hints of problems. A transect of soil cores or soil impedance readings can also indicate belowground variation.

If you apply irrigation, it must be uniform in depth. Replicates or incomplete blocks should be placed inside a basin. If you use sprinklers, irrigation must be applied when there is little wind. All sprinkler heads must throw the same amount of water, so the pump pressure must be high enough to pressurize the system evenly. Sprinkler heads must be cleaned and checked, and leaks should not occur within plots. Other management practices such as N application and weed control should be carried out uniformly as well.

If you find that uneven drying still occurs in your field, you can give a visual score of soil drying to each plot when differences are obvious, and this score can be used to adjust for field differences. Statistical designs are available that can also help deal with variability, but there is no substitute for starting with a good, uniform field.

Sec. 2

2. Know what happened

Whether you are managing irrigation or relying on natural drought periods for stress, the essential measurements you will need to characterize your environment are depth of standing water (in lowland fields), depth of the water table, and daily rainfall (see Section 2 for the importance of measuring S—the supply of water).

- The simplest measure is to record the presence or absence of standing water weekly. A late-season drought can be identified by the last date of the standing water relative to the flowering date of the variety.
- More informative is a measure of the depth of the water above and below the ground. For an accurate measure of the aboveground water, use a "slant meter"; for below the ground, use a PVC tube.
- Use a minimum of three recording stations for each trial located across any perceived water gradient.

- Make some additional measurements. It is useful to know pan evaporation and this can be measured from a central station in a region. For upland experiments, it is useful to know soil moisture tension, which can be measured inexpensively with a tensiometer (Photo 3). (For guidelines on making groundwater wells and tensiometers, see Mackill et al 1996.)
- Remember, many potentially useful data sets cannot be interpreted because nobody knows whether drought affected the experiment or not. Observations of leaf rolling in check cultivars can provide good evidence of when water stress began. It is critical to know both the dates of disappearance of standing water in lowland fields and the amount of rainfall in upland experiments. If the water table is at a depth of less than 1 to 1.5 m, it can provide an additional source of water to the crop, so check for groundwater depth. Observations of major pest and disease problems are also needed to allow interpretation of water-stress effects.

3. Keep out unwanted water

To apply stress repeatably, there must be a way to limit water input to your plots. This can be done by

- Sowing at a time of year when you expect a good chance of low rainfall (provided that this season is representative of the regular season in the target environment).
- Use a rain exclusion shelter. Rain exclusion shelters are expensive to build and maintain, so these are usually used only for small experiments. The temperature under shelters tends to be higher than the outside air temperature. This may affect crop flowering date, and can in some cases result in high-temperature damage. Monitoring of air temperature will allow you to interpret your results.
- Check for water from underground sources, especially if lowland rice is nearby. To avoid entry of water from adjacent wet areas, you need to dig between your field and the source of free water a ditch that is at least 40 cm deeper than the expected root zone. This ditch will intercept water moving into the field, and the water must then be drained away. At upland sites, lateral water movement is not usually more than 1 m or so, but, depending on the irrigation method, it may be necessary to have wider borders.

4. Remove water at the desired time

In rainfed lowland experiments, the soil is generally saturated before stress begins and the field is then drained to allow the development of drought. The number of days it takes for drought to develop depends on the moisture-holding characteristics of the soil, losses from seepage and percolation, and the amount of water transpired by the crop.

- Experiment to see when to remove water to induce stress at the desired time. Remove water at a developmental stage of a check variety. With experience, you can estimate the number of days this will require in your field. For a fully developed crop growing in a heavy clay soil at IRRI, it takes about 10 days for a field to dry from saturation to near field capacity. After about 1 week more, some leaf rolling can be observed. This means that it takes about 20 days for stress to develop after the field is drained and it would take more time if the crop were small (Photos 9–11). In contrast, sandy soils dry much more quickly and stress can develop within 14 days or so.
- In upland experiments, it will take much less time for stress to develop after rainfall or irrigation stops. If root depth is shallow (25–30 cm), the amount of water available to the crop between field capacity (about 10 kPa) and 20 kPa is only adequate for a few

days of transpiration and irrigation must be applied every 2 to 3 days in control plots. Stress will begin almost immediately on the withholding of the irrigation.

- It is also possible to apply a mild continuous stress by simply reducing irrigation frequency (Photos 6–8). A mild continuous stress has the advantage that it has a similar effect on genotypes with different flowering dates and the stress treatment is not much affected by minor rainfall events. A mild continuous stress is not very effective, however, in separating lines for some traits that require more severe stress, such as flowering delay and leaf drying.

A drought stress that reduces yield by 50% or more can provide very useful information.

5. How severe a drought stress?

Aim to reduce yield by >50%.

One reason for this is that r_G for line means estimated in trials with only slightly different stress levels is likely to be very close to 1.0. Another reason is that severe stress, when skillfully and uniformly applied, can amplify genetic differences between lines. For example, if uniform and severe drought stress can be applied to rice breeding lines at flowering, some highly susceptible lines simply do not flower. This is a large, visible genetic response that can make elimination of susceptible genotypes easy.

6. Conduct a companion nursery under well-watered conditions

In addition to the controlled-drought SE, it is very useful to have a companion nursery with well-watered conditions to estimate the yield potential of the genotypes.

- Estimate the severity of the controlled environment as the mean reduction in yield between the well-watered and the drought nursery.
- To avoid water deficit in the uplands, irrigation is usually applied when the soil moisture tension at 15-cm depth reaches about 20 kPa.
- Maintain free-standing water in the well-watered rainfed lowlands.

7. Correct for differences in flowering dates

Rice is especially sensitive to stress around flowering. This means that a line that flowers shortly after you drain your field will be much less affected by stress than a line that flowers later.

- One option is to place genotypes in early, middle, and late maturity groups and stagger the planting dates so that all genotypes flower at the same time. This requires good information on flowering time and is difficult to manage.
- Another possibility is to stratify your entries based on the flowering dates of the well-watered plots and select lines that are less affected by stress within each group.
- If there is a clear linear relationship between stress yield and flowering date, you can use a drought response index (Bidinger et al 1987). This means that you regress stress yield on flowering date in the control and find the predicted yield:

  Predicted yield = a + b (flowering date)

  The drought response index is
  DRI = (observed yield – predicted yield)/standard error of predicted yield
- Sometimes a multiple regression of flowering date and potential yield is used to calculate the DRI. However, error estimates are high and the approach discards the advantage of yield potential in the stress environment. This is best used for studies on the

Sec. 4.3

value of traits or selection of parental materials (see Section 4.3), but is not very helpful in a breeding program.

8. Other points to consider

Sec. 4.2

- Dry-season screening is, in most parts of the world, equivalent to out-of-season screening. Fields that are sown out of season are generally much more susceptible to insect, bird, and rodent attack because other food sources are unavailable. There are also climatic factors to consider, such as low temperature, high radiation, and low humidity. Because of these factors, performance in a dry-season nursery may not accurately predict yield potential for a variety targeted to the wet season. The main purpose of the dry-season nursery is to obtain additional information about drought tolerance. This information can be combined with other data from wet-season screening in a selection strategy (an example of a selection strategy for dryland wheat is given in Section 4.2).
- When rice is grown repeatedly in upland fields, yield potential often declines markedly after the first crop or two perhaps because of nematode accumulation, micronutrient deficiencies, or other unknown factors. If a field is developed as a long-term screening site, it should be large enough to allow part of the field to be rotated with a nonrice crop each year.

Additional reading

Bidinger FR, Mahalakshmi V, Rao GDP. 1987. Assessment of drought resistance in pearl millet *[Pennisetum americanum* (L.). Leeke]. II. Estimation of genotype response to stress. Aust. J. Agric. Res. 38:49-59.

Notes

Author's address: International Rice Research Institute, DAPO Box 7777, Metro Manila, Philippines.

SECTION 3.3

Experimental design and data management

G. McLaren

A major departure from conventional (irrigated) rice breeding that is required in rainfed systems is the need for early generation yield testing in selection environments that represent the TPE. Thus, here we will provide

- Details on what experimental designs to use for early generation screening trials.
- Recommendations on experimental design for advanced-line evaluations and multienvironment testing (METs).
- Information on how to manage data on germplasm, environments, and evaluations so that consistent and efficient selection decisions are made.

IRRISTAT

The software to lay out and analyze trials as described here is included in IRRISTAT. The IRRISTAT statistical package and tutorial are available on the enclosed CD or by downloading them from the Internet at www.irri.org/science/software. Simply run the setup program to install the software and copy the tutorial to your computer. The tutorials referred to above on randomization and layout, single-site analysis, and data and file management are in Microsoft Word® documents in appropriately labeled directories, or are part of the full tutorial document available in pdf format for Acrobat Reader™.

For additional reading, see Kempton and Fox (1997) and Williams and Matheson (1994) and, for new techniques of spatial analysis, Cullis et al (1998).

Early generation screening trials

In early generation screening trials, we are usually limited to very few environments; in fact, in some cases, the number of replications (r), locations (l), and years (y) may be only one. The classical methods of replication, randomization, and blocking need to be adapted to suit the purpose of screening large numbers of lines, with limited seed, space, and other resources.

Use replicate check lines in early screening nurseries

Even when all test lines cannot be replicated, one or more check lines should be replicated. Check lines in screening trials fall into two categories: probe lines that have well-known responses to specific stresses and replicated checks that may be less well known but represent the test material as accurately as possible. The check entries need not be standard varieties; they can be a random selection from the lines to be tested or a mixture of standards and test entries. The check varieties need not all be different; in fact, a single variety, repeated and then replicated, could be used. The basic assumption is that the check varieties respond to spatial heterogeneity in the same way as test varieties and that variability of the check varieties is the same as that of the test varieties. These assumptions need to be considered when the checks are selected. Some guidelines for using replicated checks are

Regularly spaced repeated checks are essential in early generation testing.

- Lay out probe lines in a systematic way. The objective of these checks is to verify that the appropriate stress was in fact applied. For drought screening, a check that is susceptible to the particular form of drought being tested should be used, and this might actually die under the applied stress.
- Identify plots for replicate check entries at regularly spaced positions in the field or screen layout. These positions must themselves be representative of the experimental space. In statistical terms, they represent a stratification of this space. They should not be selected to be at edges or along pathways or in other nonrepresentative areas, and should, in any case, be protected by border rows, plots, or pots as appropriate and necessary.

IRRISTAT

- The replicated checks should be allocated to the check plots according to a standard experimental design such as a randomized complete block (RCB) design or a Latin square design. (See IRRISTAT tutorial on Randomization and Layout.) The resulting nursery is then described as being laid out in an augmented RCB or augmented Latin square design as described below. If the field contains a single identifiable gradient, then an RCB with blocks perpendicular to the gradient is appropriate. For spatial control in two directions, the Latin square is better.

IRRISTAT

- The main objective of the replicated checks is to quantify spatial variability in the test environment and adjust the measurements of the test lines accordingly. A desirable byproduct of using replicated checks to do this is to obtain an estimate of measurement error and, indeed, if the checks themselves are interesting test material, extra valuable information is obtained on those particular lines. (See IRRISTAT tutorial on Single-Site Analysis for Variety Trials.)

Use augmented designs

Augmented designs have been developed to overcome the serious drawbacks of unreplicated trials such as a lack of control of field variability and no estimate of error for comparing entries (Federer and Raghavarao 1975). The principle of augmented designs is to embed a replicated design consisting of a few check varieties in a larger unreplicated design containing test varieties. The replicated subdesign is analyzed and used to adjust observed values of unreplicated entries for spatial effects and to estimate the error variance, which is used to compare all varieties statistically.

The use of augmented designs represents a compromise between increased control of spatial heterogeneity and reduced selection intensity. The proportion of plots allocated to check entries should be from 10% to 20% of the total number of plots available. Since we would also like to have from 12 to 20 degrees of freedom for error estimation, this places some restrictions on the size of design that can be accommodated. If some or all of the check entries are themselves test lines (we suggest using promising advanced lines for which seed is available), then of course the investment in replication pays further dividends by accurately characterizing those lines.

We describe two augmented designs:

1. *Augmented RCB designs.* The basic design plan is to divide the experimental area into several blocks across any perceived or known gradient.

With augmented RCB designs, only a few check varieties are replicated in each block, while test varieties are assigned to the remaining plots (Fig. 1). Thus, the test varieties are *not* replicated but are assigned at random throughout the blocks. Their observed values are adjusted for block differences, which are measured by the responses of the check varieties that occur in every block.

Known moisture gradient

Dry → Moist

	Block 1	Block 2	Block 3	Block 4	Block 5
1	2.5 (E07)	3.2 (E36)	4.1 (E62)	2.7 (E64)	1.4 (E04)
2	4.3 (C81)	4.1 (C83)	3.6 (C84)	3.5 (C81)	3.1 (C84)
3	4.5 (E68)	2.7 (E38)	3.5 (E49)	1.4 (E18)	2.1 (E45)
4	4.9 (E74)	2.9 (E33)	3.3 (E70)	1.0 (E17)	1.8 (E16)
5	2.8 (E09)	3.0 (E15)	2.7 (E31)	2.8 (E56)	2.1 (E51)
6	4.3 (E67)	2.3 (E30)	3.3 (E66)	3.8 (E80)	1.3 (E43)
7	4.0 (E60)	5.0 (E77)	1.7 (E20)	2.3 (E26)	1.4 (E27)
8	4.7 (C82)	3.9 (C84)	4.0 (C83)	2.8 (C84)	2.5 (C81)
9	2.7 (E19)	3.3 (E50)	3.0 (E39)	2.0 (E76)	1.1 (E06)
10	4.1 (E71)	2.4 (E12)	2.4 (E01)	2.8 (E78)	2.0 (E52)
11	4.4 (E61)	3.4 (E29)	2.9 (E37)	1.7 (E34)	1.0 (E03)
12	4.4 (E42)	2.3 (E21)	3.0 (E14)	3.4 (E79)	2.5 (E72)
13	4.3 (E69)	4.4 (E55)	1.4 (E05)	3.0 (E63)	2.3 (E57)
14	5.0 (C84)	3.9 (C81)	4.2 (C82)	3.3 (C82)	2.6 (C83)
15	3.9 (E48)	2.8 (E13)	2.4 (E11)	2.9 (E40)	1.3 (E25)
16	3.5 (E54)	4.0 (E44)	2.7 (E59)	2.6 (E53)	2.0 (E75)
17	3.2 (E02)	2.2 (E35)	3.0 (E46)	1.5 (E10)	2.7 (E73)
18	3.3 (E08)	3.6 (E65)	2.6 (E24)	3.4 (E58)	1.4 (E41)
19	3.7 (E32)	2.6 (E23)	2.6 (E47)	2.6 (E28)	1.2 (E22)
20	4.5 (C83)	4.4 (C82)	3.8 (C81)	4.5 (C83)	2.5 (C82)

Fig. 1. Yield values and line designations in the field layout of an augmented RCB design. Four checks (C81…C84) are randomly assigned to selected plots within each block (column). The eighty test entries (E01…E80) are randomly assigned to the remaining plots in the design. Blocks are arranged perpendicular to a known moisture gradient in the field.

Blocks need not all be of the same size, but, if they are, the trial is more efficient. Block size is determined by the number of blocks, the number of check varieties, and the number of test varieties. To obtain reasonable adjustments and to have sufficient degrees of freedom for error estimation, we require about five blocks and four checks. This requires 20 plots, so the design is suitable for nurseries with more than 100 plots or 80 test entries.

The analysis of an augmented RCB design is described in the IRRISTAT tutorial on Single-Site Analysis.

2. Augmented Latin square designs. Note that you can use more sophisticated augmented designs. Since the direction of field trends is unknown in most variety trials, embedding a Latin square design in an unreplicated trial is often recommended to adjust test variety yields for both row and column effects. A restriction of this design is that the number of check varieties should be equal to the number of rows and columns. An experiment should have at least five check varieties and therefore at least five rows and columns or 25 check plots to have sufficient degrees of freedom in estimating the error variance. Hence, this design is useful for nurseries with more that 125 plots or 100 test entries. An example of an augmented Latin square analysis is given in the exercise of the IRRISTAT tutorial on Single-Site Anlaysis.

Advanced yield trials and METs

The main objective of METs is to increase the number of environments where lines are evaluated. With limited resources it is preferable to increase the number of sites rather than the

Sec. 2

number of replications in any one trial. (See Section 2 for background.) To do this, we use designs that are more efficient than the RCB designs, which require many replications to reduce error caused by heterogeneity within the large blocks required to test many varieties. Lattice designs with small block sizes have been found to be effective with only two replications. Traditional simple, triple, or balanced lattices (Cochran and Cox 1957) are most efficient, but impose some constraints on the number of entries that can be tested and the block sizes that can be used. Modern alpha lattice designs (Patterson and Williams 1976) are almost as efficient and do not suffer from these constraints, but they require specialist computer programs for their design and analysis.

Some guidelines for effective METs are

- Increase the locations rather than the replications to maximize the chance of testing under drought conditions.
- Choose locations that are likely to experience the relevant drought stress.

IRRISTAT

- Use a lattice design with *only* two replications and small blocks (less than 10 plots per block) at each location. (See IRRISTAT tutorials on Randomization and Layout and on Single-Site Analysis of Variety Trials for examples of how to use classical simple lattice designs.)
- Use data from drought trials *even* if CVs are high (provided that the trial was well conducted).
- Do not use yield data from locations that do not experience the target drought stress for the TPE unless you wish to use them as an estimate of yield potential.

Information management

A robust and user-friendly information management system is an essential tool for accurate recording, documentation, and analysis of data on germplasm development, environmental characterization, and variety evaluation.

- Prepare nursery lists and field books using a system that tracks pedigree relationships between lines, including information on parentage, crossing method, location, and date of selection for each line. With this information, performance of related lines can be tracked over space and time, particularly when germplasm is exchanged between breeding projects in early generations. We recommend the use of the International Rice Information System (IRIS) and its breeder interface. (You can download IRIS and the ICIS tutorial from www.icis.cgiar.org.)
- Fully document all specific traits being measured as well as units and methods of measurement in your field book.

IRRISTAT

- Raw data should undergo data entry checks (by double entry or cross reading) and then be screened statistically for outlying values. (See IRRISTAT tutorial on Data and File Management.) This should be done as soon after collection as possible so that aberrant values can be checked or reevaluated.
- Store verified data in a central database before the appropriate analysis.

Sec. 3.2

- Use the experimental site characterization information (see Section 3.2—"Know your environment") to decide how representative each sampled environment is of the TPE.

Notes

Author's address: International Rice Research Institute, DAPO Box 7777, Metro Manila, Philippines.

SECTION

4

Breeding to improve yield under adverse environments: indirect selection using drought-tolerance traits

Section 4.1 provides some understanding on how rice responds to drought and from this identifies the most useful traits for selection (Section 4.2). We then outline the breeding approach for indirect selection for drought tolerance for different types of drought (Section 4.3) and we briefly mention the emerging use of molecular technology in breeding (Section 4.4).

SECTION
4.1

How rice responds to drought

K.S. Fischer and S. Fukai

Background theory

Numerous workers have studied the complex processes, mechanisms, and traits that determine yield of rice under moisture-limiting conditions. Fukai and Cooper (2001) have summarized this complexity and focus on three broad mechanisms that influence yield depending on the severity and predictability of the drought in the TPE—yield potential, phenology (escape), and drought tolerance. The relationship among these three components in different types of drought is shown in Figure 1. The figure shows that, at medium levels of drought stress—in which yield is reduced less than 50%—yield potential is an important mechanism for yield in the TPE. At more severe levels of drought, a mechanism for drought tolerance is required. If the drought is severe, predictable, and terminal, improvement focuses on escaping the main effects of the drought with early maturing varieties. If the drought is severe, midseason, and unpredictable, a mechanism for drought tolerance is required.

Yield potential is the upper limit of yield not constrained by water, nutrients, or pests. The determinants of yield potential are shown in Box 1.

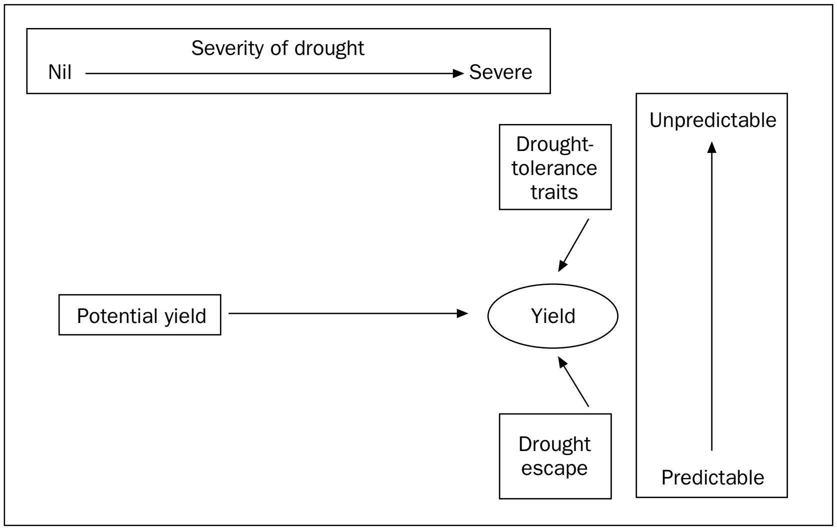

Fig. 1. Schematic diagram of three components of yield under drought-prone environments (potential yield, phenology, and drought-tolerance traits) and yield relationship in different types of drought in rainfed rice. Note: When drought is not present, yield potential determines grain production. As we move to the right in the figure, drought becomes more severe and drought escape or drought tolerance becomes more important. The vertical axis represents the predictability of drought. If drought is very predictable (bottom), drought escape through changing phenology or planting date is a good option. As drought becomes more unpredictable (moving up on the axis), drought-tolerance traits become more necessary (Fukai and Cooper 2001).

Plant breeders have improved yield potential, mainly by increasing harvest index (shorter plants and earlier flowering with more tillers and greater spikelet number), and, to a lesser extent, green leaf duration (GLD), by maintaining a larger leaf area for a longer period. Agronomists have increased radiation-use efficiency (RUE) and GLD through better nutrition of the shorter, higher-tillering plants that do not lodge with the increased fertilization.

Varieties with high yield potential will generally have an advantage over varieties with lower yield potential even with moderate drought stress (our experience suggests stress levels that result in up to a 50% loss in grain yield). The likelihood of the spillover effect of yield potential (under no stress) to mild stress environments can be estimated from the genetic correlation (r_G) of yield in the two environments. Useful spillover can be expected when the r_G is positive and significant (see Section 3).

Sec. 3

Box 1. **Determinants of yield (after Monteith 1977 and adapted from Bänziger et al 2000) and some values for rice (Mitchell et al 1998).**

Grain yield is a function of
RAD = incident radiation per day (15 to 20 MJ m^{-2} under tropical conditions)
% RI = fraction of radiation intercepted by green leaves (around 95% at the time of full canopy development, but only 45% for the crop life cycle)
GLD = green leaf duration, or number of days leaves remain green (e.g., 120 days in high-yielding varieties [HYVs] and 140+ days in traditional varieties)
RUE = radiation-use efficiency (about 2.0 g biomass [shoot] DM MJ^{-1}) under nonlimiting conditions
HI = harvest index (proportion of shoot dry matter that is grain [e.g., 0.5 in HYVs, 0.3 in traditional varieties])

Yield under water-limiting conditions is determined by the factors shown in Box 2. The production of total dry matter (DM) depends on the amount of water for transpiration and on the efficiency of water use. The grain yield then depends on the harvest index.

In rice, the most important of these components is harvest index. Drought that occurs around flowering has a large effect on spikelet fertility and thus HI and grain yield (Fig. 2).

Box 2. **Grain yield under water-limiting conditions (after Bänziger et al 2000).**

DM (biomass) = T × WUE and
Yield = DM × HI

where T is the water transpired by the crop and WUE = water-use efficiency, the efficiency of dry matter produced per unit of T.

Note: The proportion of the total available water that is transpired by the crop ranges from 0.6 for upland rice to 0.3 for lowland rice.

Thus, the main approach for breeding for drought-prone environments is to (1) improve yield potential and, depending on the type of drought, select for the appropriate combination of maturity to avoid stress during the reproductive stage and (2) select for drought tolerance of stress during the reproductive period, and avoid plant types that use a lot of water prior to flowering (i.e., produce large amounts of DM) and run out of water at the critical stage of flowering. In upland rice, as in other aerobic crops, there may also be opportunities to increase the amount of water transpired through more vigorous root systems.

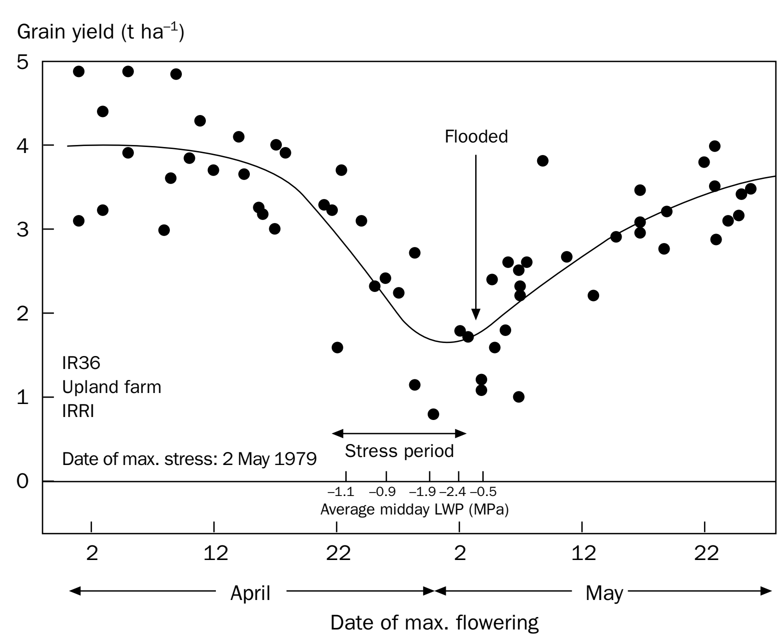

Fig. 2. Response of grain yield from the same rice variety (IR36 planted on the upland farm at IRRI) at 16 different planting dates to a single water stress period around flowering. Maximum stress occurred on 2 May. (Note the effect of timing of the stress around flowering and the large effect on grain yield.) LWP = leaf water potential. (T.C. Hsiao and O.P. Namuco, unpublished data, IRRI Annual Report 1980, cited by O'Toole 1982.)

How does rice respond to different types of drought?

The timing of drought has a major influence on how much yield loss occurs.

Early season drought

In the vegetative stage of growth, the amount of water used will be directly proportional to transpiration and thus dry matter production. The more rapid the leaf area development (i.e., greater tillering and leaf expansion), the more transpiration and the faster the use of available water. Once the canopy is full, transpiration will be determined mainly by the conductance of water through the stomata and, once they have closed, through the cuticle of the leaf. When stomata are open, both photosynthesis and transpiration are high.

At some stage, soil moisture will begin to decline and the most effective response of the plant is to reduce transpiration. In most crops (and in rice), leaf growth and stem elongation are very sensitive to water status and will be the first process affected. In most crops, other processes such as gas exchange are not affected until the available soil water has reached around 30% of its maximum value. However, in rice, there is a linear decline from around 70% of available soil water (Lilley and Fukai 1994b), indicating that rice is very sensitive to water stress. One early sign of declining soil water is *leaf rolling*, which reduces the radiation on the leaf and therefore transpiration. Leaf rolling is a simple expression of wilting (turgor loss) of the leaf (Photo 4). Another is the closing of the stomates. These responses enable the plant to reduce transpiration, conserve water, and avoid severe water deficit. However, although the rice plant may reduce water loss through closure of the stomates, considerable water may be lost through the leaf surface. Rice has a low cuticular hydraulic resistance because of limited epicuticular wax deposition (O'Toole et al 1979, O'Toole and Cruz 1983). Genotypes low in epicuticular wax may wilt earlier than normal ones under drought stress.

Sec. 4.2

Several traits are associated with these mechanisms of avoiding severe water stress and they are described in Section 4.2.

If drought occurs very early in the season, transplanting can be delayed to avoid water stress in the field. However, the transplanting of older seedlings results in greater "transplanting shock" and lower yields. Genotypes may differ in their capacity to withstand the transplanting shock of older seedlings even though the mechanisms are not well understood. Some breeding programs delay transplanting to screen for such genotypes.

If early season drought occurs after establishment, the capacity of young plants to recover is related to the amount of leaf area retained (under stress) and to their capacity for tillering after the relief of the drought stress. There is evidence of varietal differences in recovery from stress at an early stage of plant growth (Lilley and Fukai 1994b).

Intermittent midseason drought

When intermittent drought occurs around flowering, grain number and hence grain yield are affected markedly (Photo 16). Most studies show that spikelet fertility is sensitive to falling water status (see Fig. 2).

The timing of the stress relative to the developmental stage of the crop has a large effect on spikelet fertility. Varieties that differ in flowering dates by only a few days may have very different responses. This makes the comparison of genotypes of different maturity very difficult. Intermittent midseason drought occurs at any stage of development and therefore it is not feasible to avoid the critical stage of the stress by earlier maturing varieties (or different planting dates). The target is to select varieties that can avoid severe water loss (as mentioned earlier) or that can tolerate the stress with less spikelet loss (Photos 12 and 13). We describe how to select such types in Section 4.3.

Sec. 4.3

Flowering is often delayed—as much as 2–3 weeks—under drought stress (Photo 14). In some cases, the inflorescence never emerges. Panicle exsertion is related to turgor and flowering delay is negatively correlated with plant water potential (Pantuwan et al 2002). A delay in development of the female inflorescence (silking) relative to the male inflorescence (anthesis), known as the anthesis-to-silking interval (ASI), occurs in drought-affected maize. The ASI has been used effectively in plant breeding for drought tolerance in maize (Edmeades et al 1999). The use of flowering delay as a trait for selection in rice is described in Section 4.2.

Sec. 4.2

Terminal drought

Under terminal drought stress, all of the available water in the root zone will be used in transpiration. The plant will become permanently wilted and die. Yield will depend on avoiding the effect of drought around the sensitive time of flowering and on how much water can be extracted from the soil profile (Photo 2).

If the drought pattern is predictable, the best mechanism for improving yield is to escape the effects of the drought with earlier maturing varieties (or change the planting system and date). Note that flowering will still be delayed if the terminal stress begins before flowering but the goal is to identify earlier maturing genotypes under nonstress conditions and minimize the delay in flowering under the stress.

Under the terminal drought, the water extracted by the root system and the efficiency of water use determine the amount of dry matter produced (and thus grain yield). As in other crops, the extraction of soil water in rice is related to root length density and root depth (O'Toole 1982, Lilley and Fukai 1994a). Under normal soil and water conditions, rice appears to have a higher total root length than maize (Table 1). However, there is evidence that, under severe water stress, upland rice lacks the capacity to maintain root growth (i.e., an induced effect). In a comparative study of upland rice and maize, the total root length under drought stress declined by 66% and 8% for rice and maize, respectively (Kondo et al 1999), even though rice had more roots in the mild stress conditions.

Also, the pattern of distribution of roots in the soil is somewhat different in rice than in other crops. In upland rice, around 70% of the total root length was in the upper 10 cm of the soil compared with 50% in maize (Kondo et al 1999). In rainfed lowland rice, even more of the total (up to 85%) was in the upper layer (Pantuwan et al 1997). In contrast to other crops such as maize, rice is less effective in extracting water at depth in the profile. Thus, increasing root length at depth remains a trait of considerable interest in upland rice (see Section 5.3).

Sec. 5.3

In rainfed rice, there is evidence of genotypic differences in penetrating the hard pan (Samson et al 2002), but it remains to be demonstrated whether the generally low root densities at depth, as a result of increased penetration, can extract moisture and increase grain yield (Samson and Wade 1998). Wade (1999) notes that the contrast in soil conditions between aerobic drought and anaerobic flooding poses a unique set of challenges to yield improvement via manipulating root systems for rainfed lowland rice.

Table 1. Total root length (m m^{-2}) for rice and maize under various soil/water conditions.

Soil/water conditions	Rainfed lowland rice	Upland rice	Maize	Reference
Average over eight locations	8,880			Pantuwan et al (1997)
Mild stress at one location		6,980	3,495	Kondo et al (1999)
Severe stress at one location		2,277	3,306	Kondo et al (1999)

Sec. 4.2

Plants that can maintain or capture more water in the grain-filling period will maintain green leaf longer, will have less desiccation of leaf, and will have cooler leaves because of transpiration. Section 4.2 describes ways to measure these traits.

Water-use efficiency is a measure of carbon assimilated by photosynthesis per unit of water transpired. Some crops have significant genotypic variation in WUE, leading to its use in plant breeding. Extensive work on WUE in wheat indicated that this parameter is useful mainly in selection for high yield under well-watered conditions, rather than for better yield under drought stress. In rice, however, there has been little documentation of genotypic variation in WUE and its contribution to grain yield under drought is not well understood. We do not consider using WUE for selection for drought tolerance at this time.

The turgor of cells is determined in part by osmotic potential. Plants can adjust osmotic potential (i.e., osmotic adjustment) to maintain turgor under stress conditions. Osmotic adjustment (OA) can be relatively high in some rice varieties (indica), reaching 1.5 MPa when measured at 70% RWC (relative water content) (Babu et al 1999). These values are comparable with those of sorghum, which is a relatively drought-tolerant crop. Simulations of the effect of modifying OA in sorghum suggest that yield could be increased by as much as 5% under specific drought conditions (Hammer et al 1999). However, no studies have shown a relationship between OA and yield of rainfed lowland rice under drought (Fukai et al 1999).

In contrast to lowland rice, upland rice has little or no OA. To overcome this and to determine the contribution of OA to grain yield in upland conditions, efforts are under way to transfer the OA trait into upland germplasm (Courtois and Lafitte, personal communication).

Only a few traits are known to improve yield under drought.

What traits have been studied for variation in response to the different droughts?

Many traits have been studied and suggested for improving yield under the different types of drought. They are discussed in detail in the next section.

Notes

Authors' address: University of Queensland, Brisbane, Australia.

SECTION 4.2

Using secondary traits to help identify drought-tolerant genotypes

R. Lafitte, A. Blum, and G. Atlin

> *Secondary traits are plant characteristics other than yield that give additional information about how yield will change under drought.*

1. Why use secondary traits?

Grain yield under stress is the primary trait for selection in breeding programs for drought-prone environments. But it is sometimes useful to screen for secondary traits as well. Secondary traits are plant characteristics that are associated with yield under stress, and they can provide additional information for breeders to use when they make selections. Breeders who select for disease scores, plant height, and flowering date are all using secondary traits.

For a secondary trait to be useful in a breeding program, it has to pass five tests:

- It must be genetically correlated with grain yield in the predominant stress situations that occur in the target environment.
- It should not be affected very much by environment, that is, it should be highly heritable in the screening system you use.
- There must be variation among lines for the trait.
- It should not be associated with poor yields in the unstressed environment.
- It must be possible to measure the trait rapidly and economically.

Most practical drought-breeding programs emphasize direct selection for grain yield under stress. However, indirect selection for carefully selected secondary traits can be helpful in improving selection response. Selection may be for an index consisting of grain yield plus secondary or component traits, or for secondary traits only. We can predict whether the use of a secondary trait can enhance the expected progress in selection by calculating the genetic correlation (r_G) and heritability within a breeding population (see Box 1).

Box 1. How can we predict whether using a secondary trait can increase selection efficiency?

Sec. 3.1

1. Estimate heritability and genetic correlations for a population of lines. These estimates are much less useful if they are obtained from a comparison of varieties because they are strongly affected by outliers and poor adaptation of some lines. They are also not very useful if they are from a single experiment. For more details on these calculations, see Section 3.1.

   Genetic correlation (r_G): As the number of experiments increases, the normal (phenotypic) correlation begins to approach the genetic correlation. If you have data from replicated trials from at least three locations in a single year, or from replicated trials in at least two locations from two seasons, the phenotypic correlation calculated from line means will be close to the genetic correlation.

   Heritability (H): The F value for genotype in an analysis of variance can be used to estimate the repeatability of the trait:

   $$\text{Repeatability} = 1/\,(1 - F)$$

   If this term is calculated using the F value for genotype from the combined analysis of experiments across several years and locations, the repeatability is a good estimate of heritability of the trait. Remember that location is a random factor in this analysis, so the F value for genotype should be calculated as

   $$(\text{MSE for genotype})/(\text{MSE for genotype} \times \text{environment})$$

   where MSE = mean square error.

2. If the heritability of grain yield under stress is H_Y, the use of a secondary trait Z in selecting for grain yield under drought stress should be considered when $\sqrt{H_Y} < r_G\sqrt{H_Z}$.

2. When to use secondary traits

Secondary traits can improve selection response if they contribute in one of the following ways:

- Improve precision *if* the heritability of yield is reduced by stress and the heritability of the secondary trait is not reduced by stress.
- Facilitate the manipulation of the drought environment. It may be easier to reveal variation in the secondary trait than to reveal variation in yield. For example, the timing of stress has a very large effect on how much yield is reduced, so it is hard to compare lines with different flowering dates. If a secondary trait is less sensitive to the growth stage of the crop, this makes it easier to compare lines of different maturity.
- Focus the selection on a specific type of drought, whereas yield is the summation of all stresses, including those not directly associated with water.
- Are cheaper and easier to measure than grain yield under stress. Frequently, experiments are lost because of pest or weather damage before final yield can be recorded. In those cases, a good secondary trait allows useful data to be collected from the experiment.

3. What secondary traits are useful?

Secondary traits are often used in early generation testing under managed or naturally occurring drought.

Some traits appear to be associated with plant survival under water stress. If the primary effect of drought in the target environment is to kill plants, these traits may be helpful. In most places, however, the main effect of drought is to reduce grain yield without killing the plant. That is why we must find out whether there is a relationship between the secondary trait and grain yield in the target environment. But, even when we find this relationship, that is not enough to show that breeders should use the secondary trait. For breeders to use the trait, the expected progress from selection using the secondary trait and yield together must be greater than the progress made using grain yield alone. Thus, while many traits have been studied for their use in breeding for drought tolerance in rice, only a few can be recommended for use in a practical breeding program at this time. They are listed in Table 1 and described in detail below. There are other putative traits for drought tolerance (Table 2) on which research continues, but at this stage they are not recommended for application in a breeding program. The recommended traits are

- Flowering/maturity date (useful for predictable terminal drought)

  Rice is extraordinarily sensitive to water deficit from about 12 days before 50% flowering to about 7 days after flowering. If the pattern of water deficit is predictable in a given region, selection for a flowering date that does not coincide with the period of water deficit is a very effective way to improve drought tolerance. The limitations to this approach are that very early varieties may suffer a yield penalty in good seasons, and that this approach works only where the timing of the water stress is quite predictable.

  In addition to avoiding drought at critical growth stages, there may be an additional advantage to comparative earliness. Early materials sometimes tend to have a more stable harvest index than later ones.

  To measure flowering date, record the date when 50% of the productive tillers in a plot have emerged. This can be a difficult date to pinpoint, especially in stressed plots where flowering is delayed, and experienced scorers can differ by as much as 3 days in their estimates of when a plot reached 50% flowering. To improve the quality of the data, you can restrict the area to be rated to a specific central, fully bordered, part of the

Table 1. Selected secondary traits expected to be of value in some drought-tolerance breeding programs.

Trait	Relationship to stress yield	Growth stage for selection	Earliest generation for selection	Technical difficulty of selection	Heritability
Flowering/maturity date	Depends on reliability of stress timing; effective for predictable and terminal stress	Flowering	Single plants at F_2	Easy	High (approx. 0.9)
Flowering delay	High for stress at flowering	Flowering	When available seed is sufficient for a small plot stress	Easy if water can be controlled to provide uniform	Moderate (approx. 0.6)
Percent fertile spikelets	High for stress at flowering	At or near maturity	Single plants at F_2	Labor-intensive; error-prone; requires control of water	Moderate (approx. 0.6)
Leaf death score	Negative and moderate	All stages	Single plants at F_2	Easy if water can be controlled to provide uniform stress	Moderate (approx. 0.7)
Canopy temperature	Negative and fairly high if maximum stress occurs near flowering	Preflowering during full ground cover	When available seed is sufficient for a small plot	Medium	Fairly low (approx. 0.2) unless climate is very stable and vapor pressure deficit is large

Table 2. Putative traits for drought tolerance. QTLs have been identified for these secondary traits. Now they need to be tested for their relationship with performance under drought stress, and suitable high-throughput screening strategies must be developed.

Trait	Proposed function	Comments[a]	Reference
Deeper, thicker roots	To explore a greater soil volume	There is evidence from MAS that increasing root mass below 30 cm results in greater yield under stress (Section 5.3). No evidence on root thickness per se. Large-scale screening difficult.	Yadav et al (1997)
Root pulling resistance	Root penetration into deeper soil layers	Is correlated with a large root system.	Pantuwan et al (2002)
Greater root penetration ability	To explore a larger soil volume	Most studies use artificial barriers with known mechanical resistance; some controversy regarding how well this mimics the soil situation.	Clark et al (2000), Ali et al (2000)
Osmotic adjustment (OA)	To allow turgor maintenance at low plant water potential	Indica types have high OA, japonica types have low OA. This trait has been associated with a yield advantage in wheat, especially in terminal stress environments.	Lilley et al (1996)
Membrane stability	Allows leaves to continue functioning at high temperature	Genotypic differences are clear. Has been linked to heat tolerance in several species; link to drought tolerance less evident.	Tripathy et al (2000)
Leaf rolling score	Reduce transpiration	Used during vegetative stress; high heritability (approx. 0.8); but low/no association with yield. Good as indicator of stress in an experiment.	Courtois et al (2000)
Leaf relative water content	Indicates maintenance of favorable plant water status	Trait has rather low heritability; QTLs not repeatable.	Courtois et al (2000)
Water-use efficiency (WUE)	Indicates greater dry weight gain per unit of water lost by transpiration	^{13}C discrimination provides an integrated measure of WUE over the season. It has been used successfully for crops in more arid climates but has not been applied to rice.	Specht et al (2001)

[a] MAS = marker-assisted selection.

plot. This area will be more uniform and the data will be more consistent. Alternatively, if the crop is sown in hills, you can define flowering date as when a certain number of hills have produced panicles. Estimates of flowering should be recorded at least three times per week.

- Flowering delay (useful for intermittent midseason drought)

  When rice experiences a water deficit before flowering, a delay usually occurs in flowering date (Photo 14). Lines with a longer delay will tend to produce less grain, even if the water stress is relieved later. The length of the delay is partly related to how much stress the line experienced, but there is also genetic variation in how much delay results from a given level of stress. The reason for the delay in flowering is not fully understood.

  To measure flowering delay, you must have an irrigated (unstressed) control treatment sown nearby.

  Flowering delay = days to flowering in stress treatment – days to flowering in control treatment

  Make regular, reliable observations of flowering date to calculate the delay. (Because this character is the difference between two independent measurements of flowering date, the error is generally larger for the delay than for flowering date alone.) Flowering delay is best expressed when the stress is severe, so it is easily seen in fields where drying occurs over a period of weeks. In this type of stress, lines with later flowering dates will tend to be delayed more than lines that flower early because the stress intensity increases over time. To correct for this effect, you can sow lines with similar flowering dates in separate experiments and apply stress at the appropriate time for each experiment. Another approach is to make a statistical correction for flowering date. This can be done by using flowering date in the control as a covariate in the analysis.

- Percent fertile spikelets

  When stress occurs near flowering, the most sensitive growth stage, the main yield component affected is the percentage of fertile spikelets (Photo 5). The genetic correlation between yield under stress and this trait is very high, and the heritability of spikelet fertility is less affected by stress than is the heritability of grain yield. The way that spikelet fertility is affected by drought at flowering is quite specific, so it gives clearer information on genotypic response to stress than does yield, which is the integrated result of many processes that occurred over the season. However, many factors other than drought can affect spikelet sterility and some of these, such as stem borer damage, interact with drought. Experiments should be monitored for possible confounding factors.

  To measure spikelet fertility, collect a sample of representative panicles from the plot. Do not use only the tallest tillers or tillers from the main stem only; this will be strongly biased. Weigh the sample. Divide the sample randomly into two, and repeat the division until you have a subsample that is small enough to process. Weigh the subsample. Thresh the subsample by hand to remove all filled and unfilled spikelets. This cannot usually be done by rolling or other threshing methods because, if the sample is dry, the rachis will break off with the unfilled grains, or, if the sample is wet, the unfilled spikelets will remain stuck to the rachis. Separate the filled and unfilled spikelets by blowing or by flotation. Weigh the filled grains and the unfilled spikelets. Then count out 200 filled grains and record their weight, and do the same for 200 unfilled spikelets. All samples should be at the same moisture status when weighed. The percent fertile spikelets can be calculated as

$$\%\ \text{fertility} = \frac{100^{*}\ (\text{number of filled grains in the sample})}{\text{number of filled grains + number of unfilled spikelets)}}$$

$$\text{The number of filled grains is } \frac{\text{total weight of filled grains}}{(\text{weight of 200 filled grains/200})}$$

$$\text{The number of unfilled spikelets is } \frac{\text{total weight of unfilled spikelets}}{(\text{weight of 200 unfilled spikelets/200})}$$

Avoid sampling bias—make sure random representative plants are selected in each plot for measurement.

If there are large differences in spikelet fertility among lines in an experiment, you can score for this character. Some people score in the field, but there is a tendency for scorers to look only at the tallest panicles. Other groups have found that representative panicles can be collected in the field, returned to the lab, and then a scorer can individually score the panicles representing each plot. The selection of panicles to harvest is critical. The sample will be more representative if all panicles from a hill are harvested.

The problem with measuring spikelet fertility is that it requires a lot of labor and, because of the many measurements required, it is prone to error. To avoid this problem, some researchers have made visual scores of percent spikelet fertility. These scores can be used to group lines into classes of high, medium, and low fertility. Experienced scorers recommend that scoring be done on a sample of representative panicles, scoring each panicle individually rather than trying to assign an overall plot score.

Another substitute for direct measurements of spikelet fertility is the change in the panicle harvest index (PHI) with stress, where

$$\text{PHI} = \text{grain weight/weight of panicle}$$

If stress has mostly affected spikelet fertility, the support structure of panicles from stress plots is similar to that of control plots, but only part of the spikelets from stress plots form grains. This means that PHI will be lower in the stress plots. The correlation between percent fertility and panicle harvest index is quite high for rice that experiences drought near flowering.

- Leaf death (desiccation or “firing”) score

  Leaf water deficit can be further reduced beyond the point of turgor loss, reaching the point of tissue death. Leaf tissues may die (expressing desiccation) because of extreme loss of water or because of heat stress when leaf temperature rises because of inadequate transpirational cooling. Unlike leaf rolling, leaf desiccation is irreversible.

  Even though there is a gradient of water potential along the plant axis, with lower leaves having a lower water deficit while higher leaves are under a greater deficit, lower leaves often tend to desiccate before upper leaves. All leaves in the canopy should be observed when leaf death is scored. Desiccation may not occur throughout a given leaf in a uniform fashion, unless the water deficit is acute. More typically, it begins in the tip of the leaf, which is usually under greater water deficit than the basal part closer to the stem. If the timing and severity of drought in the screening environment are similar to those of the target environment, leaf drying can be well correlated with yield under stress.

To measure leaf desiccation, make a visual integration of the symptoms in a plot, based on total leaf area lost by desiccation (Photos 1 and 15). A common scoring system ranges from 0 (no senescence) to 5 (complete leaf drying). Just like for leaf rolling, it is most helpful for the final analysis if scoring is performed several times during the drought stress cycle. Because leaf desiccation is irreversible, time of day is not critical for scoring. Furthermore, since the canopy may regain turgor during the night, morning is a good time to distinguish those parts of the canopy that are indeed desiccated and dead.

- Canopy temperature

Because a major role of transpiration is leaf cooling, canopy temperature and its reduction relative to ambient air temperature are an indication of how much transpiration cools the leaves under a demanding environmental load. The relationships among canopy temperature, air temperature, and transpiration are not simple. They depend on atmospheric conditions (vapor pressure deficit, air temperature, and wind velocity), soil conditions (mainly available soil moisture), and plant characteristics (canopy size, canopy architecture, and leaf adjustments to water deficit). However, at the end of the day, breeders are interested in finding genotypes that maintain lower canopy temperature than other genotypes under the same field conditions. Relatively lower canopy temperature in drought-stressed rice indicates a relatively better capacity for taking up soil moisture or for maintaining a relatively better plant water status. Researchers have found lower canopy temperature to be correlated with final yield under stress when canopy temperature was measured near flowering (Garrity and O'Toole 1995).

This technique must be used very carefully to give repeatable results. Canopy temperature is affected by the relative amount of desiccated and dead leaves in the canopy and studies show that it can be positively correlated with leaf death score.

Canopy temperatures measured under well-watered conditions in different genotypes largely represent canopy structure variations among genotypes. For canopy temperature to represent differences in drought tolerance, measurements must be made when the population is under water deficit, as seen by some leaf rolling at midday.

Canopy temperature is measured remotely by the infrared thermometer (IRT). Canopies emit long-wave infrared radiation in proportion to their temperature. The IRT senses this radiation and converts it to an electrical signal, which is displayed as temperature. In breeding and selection work, canopy temperature is used as a comparative measure to distinguish different genotypes grown in the same environment.

To measure canopy temperature using the IRT, it is important to remember the following:

1. The correlation between canopy temperature and plant water status becomes stronger as plant water status is reduced. Therefore, measurements should be made under well-developed drought stress, typically when most of the materials in the nursery present some leaf rolling at midday. Measurements should be done around or just after midday when the plant water deficit is maximized. Since plant water status changes with the march of the day, measurements of the population must be done within about 2 hours, roughly. If a replicated test is measured, time should be partitioned between replications.

2. The thermometer has a fixed angle of view (around 2–5 degrees, depending on the model). The size of the measured target area therefore depends on the distance between the thermometer and the target. The target must consist of only canopy leaves. Any other object in the target area, such as soil surface or panicles, will result in a temperature reading that does not represent the optimal target, namely, the leaf canopy. Soil is generally hot and panicles are much warmer than leaves because they hardly transpire. Therefore, screening by canopy temperature measurements under drought stress can be done only during the vegetative growth stage, after full ground cover has been attained and before panicle emergence.
3. Since the assessment of plant stress by canopy temperature within a breeding population is relative, atmospheric conditions during measurements should be relatively stable. Cloudy or windy conditions should be avoided. Especially difficult are transient cloudiness and winds that cause vigorous flutter of the leaves.
4. The thermometer can be harmed by direct solar radiation on its lens. Viewing solar spectral reflectance from the canopy will bias temperature measurement. Therefore, readings should be made with the sun at the back of the operator, basically similar to the rule in photography. This should be taken into account when the nursery layout is planned.
5. The nursery should contain a running check cultivar, every 10 or 50 genotypes, depending on the case. Canopy temperature of the running check provides a basis for assessing site variability and offers a way to normalize data against this variability.

In selection work, breeders are interested in large differences so that they can reduce the population reliably into the most desirable materials. Experience shows that, if work is performed carefully as outlined above, a 1.5 to 2.0 °C difference can be significant (at $P<5\%$). If stress is severe and atmospheric demand for transpiration is high, genotypes may differ by up to 7 °C or more on a given day. Measurements should be made several times during the drying cycle, once or twice a week, depending on the progress of stress. For each date of measurement, data can be processed in three forms: actual temperature, temperature of the genotypes as percent of the mean temperature of the block, and temperature of the genotypes as percent of the temperature of the nearest running check. "Nearest neighbor" statistical analysis is available in the Agribase statistical software package (at www.agronomix.mb.ca/). The final data used for selection are usually derived from the day with the largest variation among genotypes, which is the date of maximum plant water deficit at peak stress.

Secondary trait data are always combined with yield data for selection.

4. How can secondary traits be used in real life?

Secondary trait selection does not replace yield selection under drought stress unless stress is severe enough to reduce yield to an insignificant level. The usual approach is to combine yield data from relatively low-stress or well-watered conditions with yield and secondary trait data derived from a stress nursery. This multiple data for selection will be superior to direct selection (for yield) in cases in which H for yield is low, H for the secondary trait is high, and r_G is high.

Many designs combine multiple data sets into a selection index for use in a breeding program. These are useful and important for laying out the theoretical basis for selection practices in plant breeding, especially for complex quantitative traits such as yield. They also offer breeders statistically sound methods of selection based on the theory of quantitative genetics.

However, most active breeders do not use the rigid statistical selection indices to process their own nursery data for decision making toward selection. Most breeders acknowledge the importance of such indices but tend to apply their own simpler and more flexible decision systems.

However, breeders prefer a system that is built on quantitative genetics principles, but is flexible enough to allow them to apply their own experience and knowledge of the selection environment and even their intuition. Breeders most often use a system of multiple cutoffs. They might first identify all lines that yield as well as the check and then eliminate any lines that have very poor drought scores, lines that have a flowering date outside the desired range, etc. A detailed example of how data can be combined from control and stress nurseries to make final selections is given in the wheat breeding example in Box 2.

Simple spreadsheets can be used for selecting lines using multiple data sets.

Sec. 4.3

Box 2. An example of the use of multiple data for yield and drought tolerance in selection (for wheat). Source: A. Blum.

The following example presents a simple and flexible approach to achieve selection by considering several criteria and their desirable thresholds, at will. The method is based on an MS-Excel worksheet and its data autofilter utility.The example involves real data from a wheat breeding program where selection among 255 advanced F_6 lines is performed. (See Figure 1 and Box 1 of Section 4.3 for details of the breeding program.)

Note in the screening process described in Figure 1 and Box 1 (Section 4.3) that selection has already been performed on F_2 to F_4 to optimize, in a broad sense, plant height, tillering rate and synchrony, resistance to extreme lodging, presence of awns, disease reactions, and grain quality. In this specific case, heading date was not optimized during early generation selection. Thus, in this example, we are using the following multiple data set, which is appropriate for the selection of drought tolerance in wheat. The multiple data set includes

- IrrYld—yield under well-watered (potential) conditions. Mean nursery yield was 768 g m^{-2}.
- IrrYld%Chk—yield under well-watered conditions of each entry as percentage of the control (check), which is the standard dryland cultivar of choice.
- DryYld—yield under preflowering drought-stress conditions where mean nursery yield was 35.5% of the mean yield of the well-watered nursery.
- DryYld%Chk—yield under drought conditions of each entry as percentage of the check cultivar.
- HeadDays—days from emergence to heading under well-watered conditions.
- Temp%—midday canopy temperature (taken with the infrared thermometer) at peak stress, which occurred just before heading, expressed for each entry as percent of the mean temperature of the block.[1]
- FillInj—This is a measure of wheat capacity to fill the grain from stored stem reserves in the absence of any assimilation during grain filling, as achieved by chemical desiccation of the canopy. It is expressed as percent reduction in mean kernel weight when the canopy was desiccated with magnesium chlorate spray at the onset of grain filling, as compared with the nontreated control (Blum 1998).
- SED—Sedimentation is a fast screening test for wheat baking quality. Higher sedimentation is associated with better bread baking quality.

On the spreadsheet, the headings of the above variables as they appear on the first row are selected. Then the Autofilter option is selected from the Data menu. A drop-down menu marked by an arrow appears on each variable heading (Fig. 2). The threshold is set by clicking the arrow on the selected variable, which opens a menu for the various threshold options.

- The first step is to select all entries that yielded more than 100% of the check variety. This is done by clicking the arrow on IrrYld%Chk and choosing "custom" and setting it for "greater than" and "100" (Fig. 3). One might choose to set the threshold appropriate to the magnitude of the error or LSD for the given variable, for example, greater than 104% if 4% was the error.

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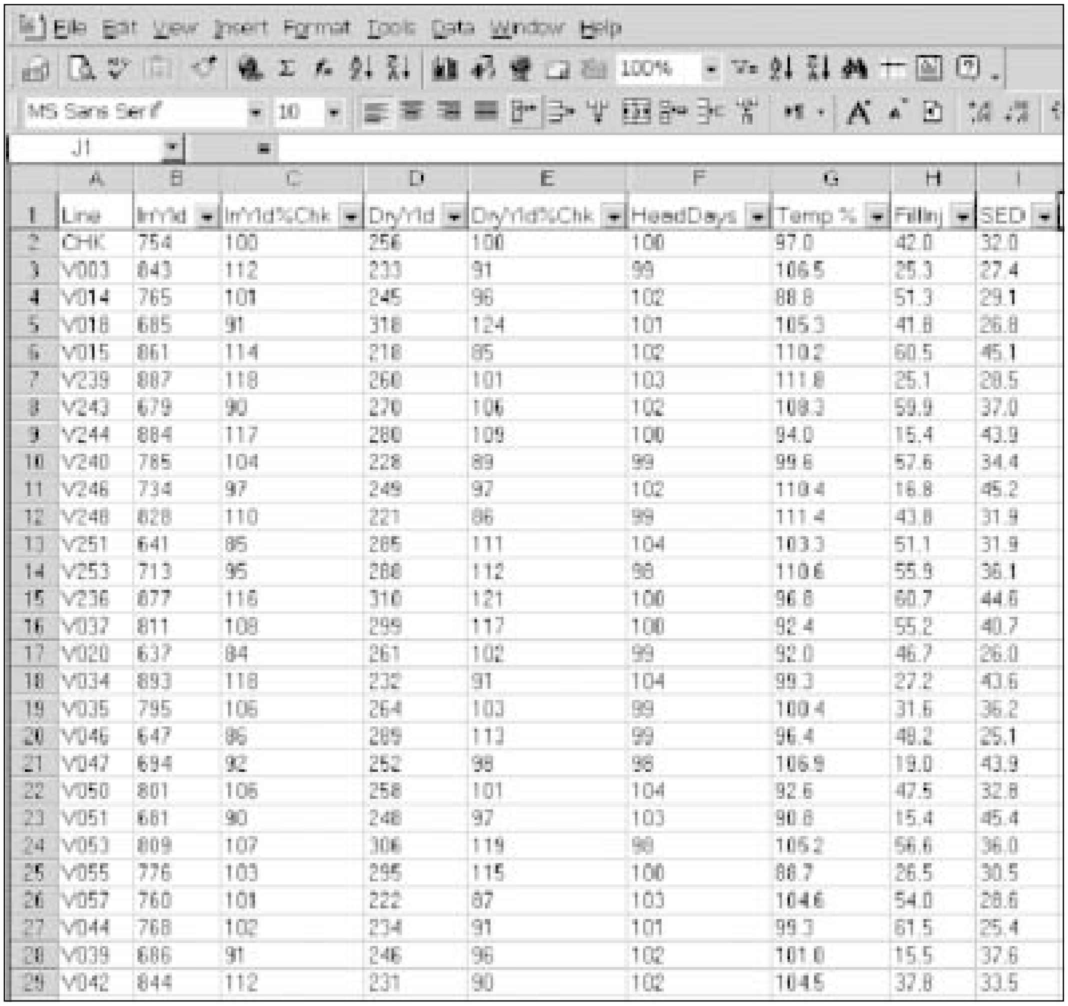
[1] Web site: www.plantstress.com. Select "Files and presentations/this site" and see the file "Leaf canopy temperature and its measurement in the field."

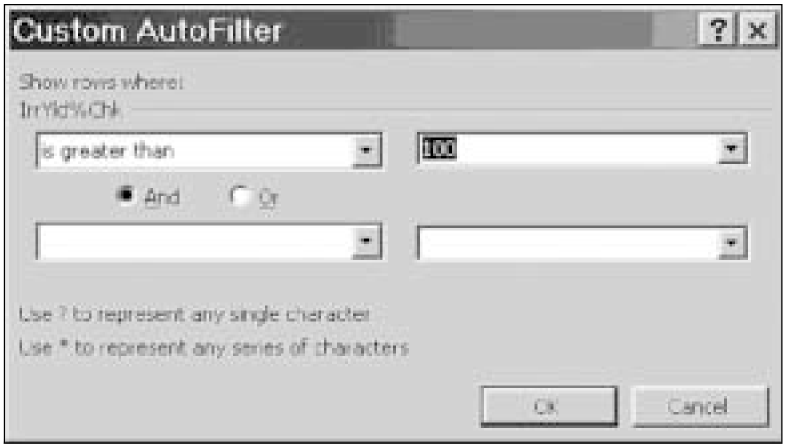


	A	B	C	D	E	F	G	H	I
1	Line	IrrYld	IrrYld%Chk	DryYld	DryYld%Chk	HeadDays	Temp %	FillInj	SED
2	CHK	754	100	256	100	100	97.0	42.0	32.0
3	V003	843	112	233	91	99	106.5	25.3	27.4
4	V014	765	101	245	96	102	88.8	51.3	29.1
5	V018	685	91	318	124	101	105.3	41.8	26.8
6	V015	861	114	218	85	102	110.2	60.5	45.1
7	V239	887	118	260	101	103	111.8	25.1	28.5
8	V243	679	90	270	106	102	108.3	59.9	37.0
9	V244	884	117	280	109	100	94.0	15.4	43.9
10	V240	785	104	228	89	99	99.6	57.6	34.4
11	V246	734	97	249	97	102	110.4	16.8	45.2
12	V248	828	110	221	86	99	111.4	43.8	31.9
13	V251	641	85	285	111	104	103.3	51.1	31.9
14	V253	713	95	288	112	98	110.6	55.9	36.1
15	V236	877	116	310	121	100	96.8	60.7	44.6
16	V037	811	108	299	117	100	92.4	55.2	40.7
17	V020	637	84	261	102	99	92.0	46.7	26.0
18	V034	893	118	232	91	104	99.3	27.2	43.6
19	V035	795	106	264	103	99	100.4	31.6	36.2
20	V046	647	86	289	113	99	96.4	48.2	25.1
21	V047	694	92	252	98	98	106.9	19.0	43.9
22	V050	801	106	258	101	104	92.6	47.5	32.8
23	V051	681	90	248	97	103	90.8	15.4	45.4
24	V053	809	107	306	119	98	105.2	56.6	36.0
25	V055	776	103	295	115	100	88.7	26.5	30.5
26	V057	760	101	222	87	103	104.6	54.0	28.6
27	V044	768	102	234	91	101	99.3	61.5	25.4
28	V039	686	91	246	96	102	101.0	15.5	37.6
29	V042	844	112	231	90	102	104.5	37.8	33.5

Fig. 1. Top part of a spreadsheet containing wheat nursery data for eight variables and the set autofilter menu for each variable (see text).

- DryYld%Chk >100, selecting all entries yielding better than the check variety under drought stress.
- HeadDays <106; early heading is important. The check variety, which is quite early in heading, stands at 100 days
- Temp% <100%; since cooler canopies under stress represent better plant water status, lines with temperatures lower than the mean of the block are selected. One may choose to select lines cooler than the check cultivar, for example.
- FillInj <45%. The check variety scored 42.0%. The range was 15.1% to 60.0% but many of the low-injury entries were later in heading than 106 days and were already excluded after filtering for HeadDays. Here, one must weigh the importance of grain filling from stem reserves against maximizing earliness of heading. If at this point one filters HeadDays for a threshold of, say, 108 days, then more entries with low FillInj can be recovered.
- SED >29. The check variety, which has satisfactory bread baking quality, has SED = 30.

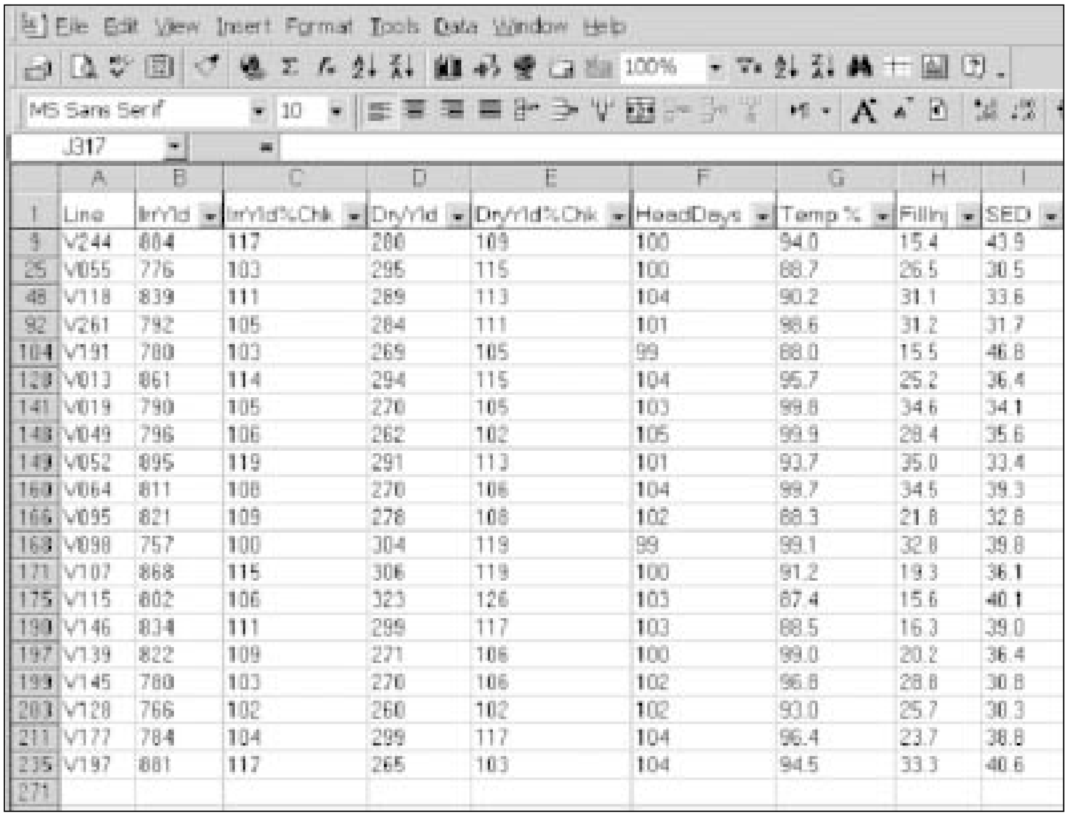
The selected lines out of a total of 255 are presented in Figure 3. In viewing these results, breeders have the option to modify the set thresholds and revise the selection (filtering) process. For example, one may decide to increase the number of final selected lines by reducing the threshold for yield under well-watered conditions (say, IrrYld%Chk >98). Breeders there-



Show rows where:
IrrYld%Chk
is greater than 100
● And ○ Or

Use ? to represent any single character
Use * to represent any series of characters

Fig. 2. Example for setting an autofilter (see text).



	A	B	C	D	E	F	G	H	I
1	Line	IrrYld	IrrYld%Chk	DryYld	DryYld%Chk	HeadDays	Temp %	Fillin	SED
9	V244	804	117	280	109	100	94.0	15.4	43.9
25	V055	776	103	295	115	100	88.7	26.5	30.5
48	V118	839	111	289	113	104	90.2	31.1	33.6
92	V261	792	105	284	111	101	98.6	31.2	31.7
104	V191	780	103	269	105	99	88.0	15.5	46.8
128	V013	861	114	294	115	104	95.7	25.2	36.4
141	V019	790	105	270	105	103	99.8	34.6	34.1
148	V049	796	106	262	102	105	99.9	28.4	35.6
149	V052	895	119	291	113	101	93.7	35.0	33.4
160	V064	811	108	270	106	104	99.7	34.5	39.3
166	V095	821	109	278	108	102	88.3	21.8	32.8
168	V098	757	100	304	119	99	99.1	32.8	39.8
171	V107	868	115	306	119	100	91.2	19.3	36.1
175	V115	802	106	323	126	103	87.4	15.6	40.1
190	V146	834	111	299	117	103	88.5	16.3	39.0
197	V139	822	109	271	106	100	99.0	20.2	36.4
199	V145	780	103	270	106	102	96.8	28.8	30.8
203	V128	766	102	260	102	102	93.0	25.7	30.3
211	V177	784	104	299	117	104	96.4	23.7	38.8
235	V197	881	117	265	103	104	94.5	33.3	40.6
271									

Fig. 3. The results of selection by filtering eight variables over 255 wheat lines.

fore have the freedom and flexibility to modify the selection process according to their opinions, knowledge of the specific test conditions, and results of the actual selection process on the spreadsheet.

5. How can you decide whether a secondary trait adds value to your breeding program?

In a previous section, we described two requirements of a good secondary trait: high heritability and good correlation with yield under stress. These values will provide useful predictions for breeders, but we also like to see a direct demonstration of the value of a trait. How can you test the real value of a secondary trait?

- Make an experimental variety by recombining lines with good levels of the secondary trait. Then compare the yield of the experimental variety under stress with the average of the population under stress. This is a time-consuming approach, and so far it has not been used much in rice.
- Use a mapping population (if available) to identify subsets of lines with high versus low levels of the secondary trait. These subsets can then be tested for differences in grain yield under stress.
- Identify QTLs for the trait and then see if the QTLs for yield under stress are also found at the same places (that is, if there is cosegregation of QTLs). A secondary trait may be directly related to yield through a physiological mechanism or it may be a genetic marker that is closely linked to yield-related genes. Either of these cases would be revealed by cosegregation of QTLs. However, this approach has several problems and these are discussed in Section 4.4.

Sec. 4.4

6. Are there other putative traits for drought tolerance?

We list several putative traits that have been studied for their role in drought tolerance in Table 2. Some scientists think that a trait might be useful as a selection criterion if it improves an intermediate process such as plant water uptake. They argue that another limiting process may "mask" the importance of the trait to yield. These putative traits are hypothesized to be of value on the basis of our understanding of crop physiology or biochemistry. Most such traits, however, cannot yet be recommended as part of an ongoing breeding program for drought tolerance, particularly if they are expensive or difficult to measure. However, some can be used for the selection of parents.

One of these traits, the leaf rolling score (Photo 4), is very useful for the purpose of recording when the crop begins to be stressed. Rolling occurs when the cells lose turgor and the leaf wilts, and it is a very clear visual symptom of plant water deficit. In general, if a certain cultivar does not show leaf rolling while others do, this is an indication that this cultivar has a relatively better water status. That may be a result of deep roots that allow continued water uptake, effective osmotic adjustment that maintains turgor at a given leaf water status, or less leaf area and slower water use. Because rolling can reflect many different mechanisms, it is not generally correlated with yield under stress. But you can use records of leaf rolling in check varieties to know when the crop began to experience stress, or whether certain areas of the field were suffering from more drought than other parts. As leaves stop growing and mature, they generally tend to lose the capacity for rolling. This is typical of older leaves in plants that have reached or passed the flowering stage. When older leaves do not roll and water is not available for transpirational cooling, their temperature can rise excessively and they may die. This is one of the reasons for severe leaf desiccation and "firing" under drought stress (see above). Therefore, leaf rolling is a reliable index of stress, mainly preflowering. Leaf rolling is scored on an arbitrary scale of 0 (no rolling) to 5 (tight rolling). Attempts were made to graphically design a rolling scale, but breeders can decide on their own scale for visually integrating the tightness of rolling and the relative amount of leaves rolled in the canopy.

Leaf rolling is usually reversible. Plant water deficit generally increases over the day, with a maximum around solar noon. Therefore, rolling scores may change during the day, being lowest in the morning and greatest around or just after solar noon.

To measure leaf rolling, use the IRRI scoring system (IRRI 1996). Make your readings at or around noon. Repeat the scoring during each drying cycle for one or more running check cultivars, and these data can be useful for normalizing the final results (see discussion of canopy temperature). This is especially important when the nursery site is not homogeneous for water stress development.

Notes

Authors' addresses: R. Lafitte and G. Atlin, International Rice Research Institute, DAPO Box 7777, Metro Manila, Philippines; A. Blum, PO Box 16246, Tel Aviv 61162, Israel.

SECTION
4.3

Indirect selection for physiological traits for drought tolerance

D.J. Mackill, S. Fukai, and A. Blum

This subsection will describe how to incorporate selection for secondary traits for drought tolerance into a conventional breeding program based on selection for grain yield as described in Section 3.

1. Defining the selection strategy for the TPE

The earlier sections have described how to define and characterize the TPE based on water supply and types of drought. Although the TPE and the approaches to develop drought-tolerant varieties will be specific to your own conditions, the following are some of the more common TPE (for drought) and approaches for indirect selection. We use them to provide examples to incorporate specific selection for drought tolerance in your own breeding program. However, it is important that you develop your own improvement strategy for your own TPE.

Sec. 4.1

We use the conceptual model for yield under drought as shown in Figure 1 in Section 4.1, that is, that yield under drought is a function of (1) yield potential, (2) the flowering date to escape the drought, and (3) traits that provide drought tolerance. The breeding strategies for the examples that follow use different combinations of these components. In all cases, indirect selection is in addition to direct selection for grain yield as described in Section 3.

2. Indirect selection for a TPE with predictable late-season drought in rainfed lowland rice

This TPE is characterized by

- A predictable late-season drought, such as what occurs in geographic areas of short-season but reliable rainfall.
- Yield in the target area is highly correlated with yield potential (i.e., yield under well-watered conditions) at mild and medium stress (i.e., up to 50% yield loss).
- There is an optimum flowering date and maturity date that are usually earlier than those of the local material.

A plant breeding strategy to develop varieties adapted to the TPE of predictable terminal drought would include

— Direct selection for yield in the target environment
— Selection for yield potential (under irrigated conditions)
— Selection for optimum flowering date
— Note that there is no selection for traits specific to drought tolerance.

The approach is to

- — Measure yield potential by growing a nursery under irrigated conditions at a site in the target domain. If there is no irrigation facility, locate a test site in the most favorable positions (rainfall and toposequence) in the TPE to obtain the best expression of yield. Monitor the depth of the water table and use data from only those sites that have had adequate water through the entire season to estimate yield potential.
- — Measure days to flower in the well-watered plots. Plotting the relationship between flowering date (of the well-watered plot) and yield in the TPE will establish an optimum maturity date. This is usually about 15 days before standing water disappears at the end of the wet season at the TPE sites.
- — Use a managed-stress environment for the terminal drought. Remove water from the field to have stress develop in mid grain filling. Record the level of the water table and measure plant water status in the check entries.
- — Yield-test in the TPE. Record the level of water in the field. To facilitate selection of the earlier, high-yield-potential lines, separate the lines into groups based on when they flowered in the well-watered treatment. Select for yield in the desired flowering group.
- — Begin progeny yield testing in an early generation (as early as F_3) in the program.

3. Indirect selection for a TPE with intermittent mid- to late-season drought in rainfed lowland rice

Choose the best selection strategy for the type of drought in the TPE.

This TPE is characterized by

- A moderate to high frequency of drought occurring around flowering.
- Moderate to high (i.e., greater than 50%) yield loss.
- Drought occurrence is unpredictable.

Such a TPE might represent geographic areas of low and variable rainfall and areas at the top of the toposequence where there is less water.

A plant breeding strategy to develop varieties for this TPE and type of drought includes

- — Direct selection for yield in the TPE.
- — Selection for yield potential (under irrigated conditions).
- — Selection for yield under managed-drought trials (modify the test environments to simulate drought of the target domain).
- — Selection for flowering delay, spikelet sterility, and other drought traits (leaf rolling, desiccation) of proven contributions.

The approach is to

- — Measure yield potential as discussed earlier.
- — Use a managed-stress environment for the midseason drought. Plant on the upper part of the toposequence and remove water from the field to allow stress to develop by flowering. Record soil moisture status and measure the relative water content or leaf water potential in the check entries.
- — Measure days to flower in the well-watered and drought plots with maximum precision in order to calculate the flower delay. (If necessary, deploy field technicans by replications so that any bias in their estimate of 50% flowering can be taken out in the ANOVA.)
- — Measure spikelet fertility in the managed-drought trial.
- — Yield-test in the TPE. Record the level of water in the field. To facilitate selection of the earlier, high-yield-potential lines, separate the lines into groups based on when

they flowered in the well-watered treatment. Select for yield within each of the flowering groups.

— Begin progeny yield testing in an early generation (such as the F_3) in the program.

Box 1. **Incorporating selection for stress adaptation in a wheat breeding program. Source: A. Blum.**

Background

The wheat breeding program at the Volcani Center (Israel) aims to improve yield under the typical Mediterranean dryland conditions of the region. The breeding program has a long history of selection for grain yield, grain quality, and disease resistance using a conventional approach. Recently, it began integrating several supplemental stress-resistance selection criteria directed at improving adaptation to dryland conditions. This has been implemented without compromising the ongoing selection for yield, grain quality, and disease resistance.

In principle, early generation selection was designed to conserve the desirable ideotype in terms of phenology, plant development, lodging resistance, disease resistance, and grain type. Selection for specific stress resistance and dryland adaptation traits was performed at the more advanced generations of the agronomically acceptable genotypes. There is also sufficient seed of the later generations to conduct all of the trials to select the multiple traits.

Note: This example in wheat shows how screening for multiple traits can be done in a routine breeding program. We advocate the approach for rice, not the traits per se.

Screening for multiple traits

Figure 1 shows a schematic general outline of the program flow, which can be modified depending on materials, seasonal problems, and size of populations. The selection criteria used for improved stress resistance and dryland adaptation employed in Figure 1 include

- Early flowering as an important drought escape mechanism in the Mediterranean region (Blum 1988). Selection is made in the F_2 and F_3 for optimal flowering rather than extremely early flowering. The long-term yield performance trials in the region indicate that the desirable heading date is around 10 days earlier than for typical CIMMYT (International Maize and Wheat Improvement Center) wheat materials such as Veery.
- Presence of awns (e.g., Olugbemi and Bush 1987) selected for in the F_2 and F_3.
- Yield under nonstress conditions. Tests are performed in the F_5 and later generations in a region with a mean annual rainfall of 550 mm and with supplemental irrigation and complete weed and disease control. The yield of selected lines is equal to or above that of several standard dryland cultivars.
- Yield under stress (dry) conditions. Tests are performed in the F_6 and later generations in a region with a mean annual rainfall of 240 mm. The most common stress occurs at or after heading. On some occasions, however, there is intermittent early stress from which the plants have recovered. If drought during this early vegetative stage is too severe without a foreseen recovery by rainfall, then some irrigation is applied to bring about a recovery so that yield can be determined.
- In the above test, and when stress occurs before heading, data are recorded on leaf rolling score, leaf death score, and midday canopy temperature (Blum et al 1982, 1990), at or close to the peak of the stress.
- In a special nursery under well-watered and disease-free (controlled) conditions, the F_6 and F_7 lines are chemically desiccated. This test (Blum 1997, 1998) is designed to reveal the capacity for grain filling from preheading stored stem reserves. This is a crucial mechanism supporting grain filling under drought, heat, and disease stress during grain filling.
- In the Mediterranean region, wheat often undergoes moderate chronic heat stress during the second half of the growth cycle. Genotypes from the F_6 and F_7 lines that are less affected by these temperatures are selected by their performance in an off-season summer nursery where conditions are very hot. The lines are grown under full irrigation (Shpiler and Blum 1986). Plant growth and yield generally decline to 40–50% of what they are in normal winter growing conditions. Selection is performed mainly for yield, grain shriveling, and a visual general plant vigor score at heading.

In the advanced generations, the selected seed is taken from the nonstress trial, based on information obtained in all tests. The accumulated data in the different tests are used to construct a flexible selection index, described in the next section.

4. How do you integrate the additional selection criteria in an ongoing drought-breeding program?

The generic steps for a plant breeding program based on yield selection and other traits for pest resistance, grain quality, etc., are described in Section 3 and in Mackill et al (1996).

We focus here on the additional steps for incorporating traits for drought tolerance into the more routine breeding program. In some cases, you may need to modify your current breeding and testing system to accommodate the additional selections as described in the case study of the Thailand breeding program (Section 5.1).

Another illustrative example is the wheat breeding program of the Volcani Center, Israel, as shown in Box 1.

Each breeding program will have specific needs and constraints. The following are guidelines to assist you in developing your breeding program for:

1. Parental selection and hybridization

As with all breeding programs, progress will be greater with the use of parents that have demonstrated yield superiority in the target domain. To select the best parents,

- Use farmer participatory assessments to help identify the complementary traits required in the parents. Participatory varietal selection greatly aids the selection of parents by identifying both germplasm and required traits (Witcombe and Virk 2001). The objective is to obtain segregating populations allowing maximum progress for selection of yield in the TPE and for the material to be acceptable to farmers and end-users. Although drought tolerance is the major concern in this manual, the selec-

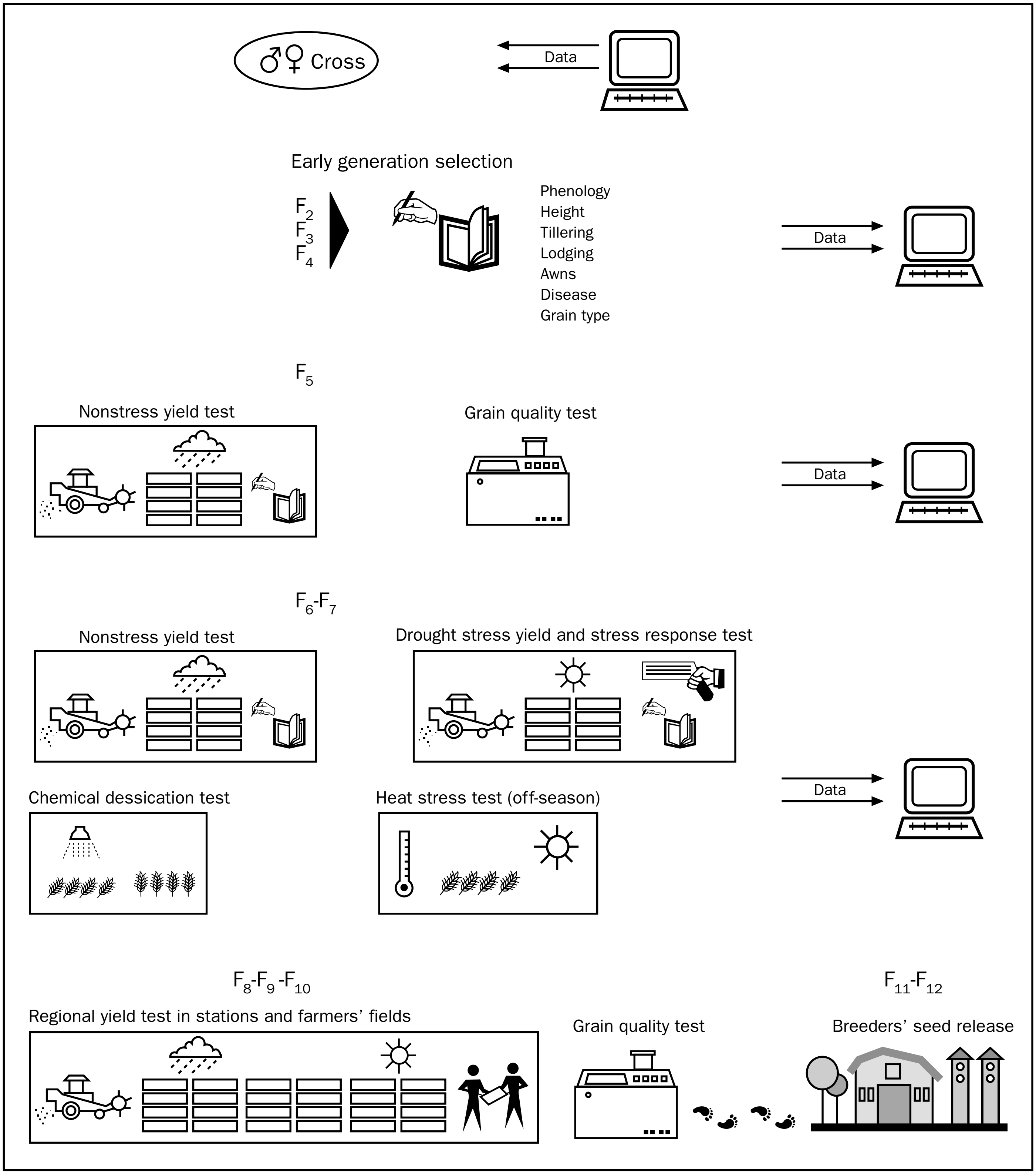


Fig. 1. Schematic representation of the wheat breeding program at the Volcani Center, which incorporates several selection criteria for improved adaptation to dryland conditions. See text for explanation.

tion of parents will take into consideration all important traits required in the region, such as grain quality, weed competitiveness, and pest resistance.

- Use materials with high yield potential (usually exotic) and with the optimum maturity date.
- If no drought-tolerant cultivars are known, evaluate a diverse range of cultivars and advanced lines for the characters identified for the TPE, including the specific characters for drought tolerance. This will mean testing the potential parental material under controlled drought.
- Use a DRI (drought response index) to normalize "the effects of yield potential and flowering date" on yield under drought stress. DRI ranges from –2 to 2 and values greater than 1.4 may be considered as drought tolerance. When several experiments are considered, the mean DRI of the drought-tolerant genotype may be below 1.4, with the actual value depending on the consistency of performance across the experiments. The DRI provides a better estimate of the contribution of drought-tolerance traits to yield under drought independent of those for yield potential and flowering. This estimate is, however, prone to high errors, and it should be considered mainly as supporting evidence.
- Because of the complexity of the genetics of drought tolerance, one useful strategy is to backcross simply inherited traits into a drought-tolerant cultivar. Cultivars that are widely grown in a particular region are probably prized for their yield stability (and therefore possibly their drought tolerance, as well as other desirable traits such as grain quality). The studieswill probably identify parental material with drought tolerance from among these materials. In some cases, these cultivars can be improved through the introduction of a few genes—disease resistance, shorter plant height, and early maturity—by backcrossing.
- However, in most cases, the parental material for crossing will be chosen for the adaptation and drought tolerance from one parent and for the high yield potential from the other parent. (A detailed description of the selection of parental material is given in Sections 5.1 and 5.3.)

Sec. 5.1
Sec. 5.3

2. Early generation selection

In Section 3, we highlighted the need to conduct as many trials at as many sites as possible for yield in the SE of the TPE and as early as possible in the population improvement process.

The aim of the breeding program is then to develop fixed lines for early yield testing at a large number of sites (direct selection for yield) and under controlled drought conditions (indirect selection).

- Fix lines through single-seed descent (SSD). The main goal is to fix the lines with minimum selection. Where facilities are available to control daylength (and when using photoperiod-sensitive materials), up to three generations can be produced (using the rapid generation advance, RGA) per year, thus reducing the time to develop fixed lines (F_5 and later) for yield testing.
- Fix lines through the normal process of single plant selection within the F_2 and later generations in the bulk method. Usually, two generations are developed each year by the use of an off-season nursery. This provides an opportunity to select for characters that are more highly heritable (selection is based on a single plant or progeny row and one observation). It also creates a danger that selection, particularly under irrigation or

in the off-season nursery, will not be representative of the TPE. (These two approaches are described in detail in Section 5.1, where some guidelines for the size of the population to be evaluated are also given.)

- Select for traits such as maturity and height (main season) and disease resistance only in the early generations if the desirable agronomic traits have been identified with farmers' priorities in mind. For example, breeders may select short materials because of their high yield potential, but these may not be accepted by farmers because of various problems such as poor weed competition and low straw yield.
- Select under drought conditions in the early stages. Many plants in a segregating population may not produce any seed because of susceptibility to drought. Since the heritability of drought tolerance is usually low, it will be beneficial to practice this type of selection for more than one generation. Many breeders find that the bulk method of breeding is suitable for this type of environment and requires fewer resources than the pedigree method (Mackill et al 1996).

3. Evaluation of fixed lines, including data collection and final selection

When fixed lines are developed (F_5 or later), seed supplies are sufficient for replicated testing. This will allow more flexibility in conducting METs in the TPE. (Section 5.1 gives an example of the evaluation process, with an approximate size of the populations required to make progress.)

Sec. 3

Sec. 5.4

Sec. 5.1

- Maximize the number of test locations that are representative of the TPE, including the managed-stress environment (for details, see Section 3).
- Use a simple selection index to incorporate all of the additional data. In practice, however, these indices are not used in plant breeding programs. Decisions as to the relative weights are generally left to breeders' intuition, and these should reflect the relative priorities of farmers. This emphasizes the importance of participatory approaches in all rainfed breeding programs. A simple spreadsheet approach to the selection of a range of characters is described in Section 5.4.
- Use new tools in the analysis of the large amount of data and in the final selection. In the case of multiple data sets for yield (including the drought-manipulated site), we recommend the use of pattern analysis of GEI as described in Section 5.1. This allows the grouping of a large number of genotypes into "clusters" of similar response. The additional data on other characters can then be used to select for families within the desired groupings.

Notes

Authors' addresses: D.J. Mackill, International Rice Research Institute, DAPO Box 7777, Metro Manila, Philippines; S. Fukai, University of Queensland, Brisbane, Australia; A. Blum, PO Box 16246, Tel Aviv 61162, Israel.

SECTION
4.4

What molecular tools are available for selection for drought tolerance?

D.J. Mackill

In this section, we briefly review the status of marker-assisted selection (MAS) for breeding for drought and suggest companion studies to the routine breeding program that will facilitate the use of MAS in the future.

The use of DNA markers to identify genes of economic importance has become a goal in many rice research programs. These markers provide an unprecedented ability to uncover the underlying genetic control of important phenotypic differences. The DNA markers can be used to screen large numbers of germplasm materials for a particular trait and thus assist with the conventional breeding process. Despite the potential of these markers, however, examples of their successful integration into applied breeding via MAS are rare, and even more so for screening for drought tolerance.

Sec. 4.2

Sec. 5.4

As of the writing of this manual, it is not possible to recommend immediate application of molecular markers to develop improved drought-tolerant rice cultivars. Although many quantitative trait loci (QTL) have been mapped for traits associated with drought response (Mackill et al 1999, Nguyen et al 1997, and see Table 2 in Section 4.2), particularly root characteristics, the relationship between these loci and drought tolerance, measured as relative yield under drought stress, is not well established. Section 5.4 presents an example of MAS applied for root depth under upland conditions. It is expected that current research will identify more candidates for marker-assisted selection; therefore, it is useful to consider some of the practical steps in employing MAS in a breeding program for drought tolerance. The case study of MAS for selection for roots in upland rice (see Section 5.4) provides a practical application of these steps.

At this time, there are no practical molecular markers to assist breeding for drought tolerance in rice.

1. When can we use MAS?

The most common situations in which MAS will probably have an advantage are

- when the phenotype is expensive or difficult to measure accurately,
- when multiple genes conferring a similar phenotype are being combined, and
- when there is a need to rapidly remove donor chromosome segments in a backcrossing program.

The last two cases generally apply to the introgression of major genes such as those conferring disease resistance and may not be as relevant to drought tolerance, although markers could be used to remove donor segments not associated with drought-tolerance QTLs during MAS.

The ultimate objective of QTL mapping for drought tolerance is to be able to identify multiple QTLs for transfer into more productive breeding lines. For example, Mackill et al (1999) outlined a procedure to transfer several root-related characters into improved rainfed lowland cultivars. One must be careful, however, to limit the number of QTLs to the most important ones because MAS is not very effective when the number of QTLs is more than three or four. The population sizes needed for MAS increase rapidly with the addition of more QTLs.

2. How can we develop more applications of MAS?

The identification of loci conferring drought tolerance will come through both QTL mapping and the newer techniques of functional genomics and proteomics. QTL mapping studies must focus on direct measurement of drought tolerance, rather than mapping traits thought to be associated with the trait. The challenge is that it is very difficult to measure drought tolerance for a large number of genotypes. This type of experiment can be done using only homozygous mapping populations such as doubled-haploid (DH) populations or recombinant inbred lines (RILs) or near-isogenic lines (NILs).

- Produce mapping populations of your breeding material. The best approach is to use an F_1 of a cross between two diverse parents that differ in drought tolerance. Genetic diversity is needed to ensure that there is sufficient marker polymorphism for mapping. A DH population can be produced rapidly and should be highly homozygous with low segregation distortion. However, many researchers do not have adequate anther culture facilities and many F_1 combinations have a poor response to tissue culture.
- The RIL approach is easier, but RIL populations take several years to produce, and the resulting populations may have high segregation distortion (Wang et al 1994).
- The QTL mapping method is necessary to identify the cause of genetic differences for drought tolerance between genotypes. It is important, however, that large enough populations be used. At least 200 lines are required, but up to 500 would be needed to identify QTLs of smaller effect (Lande and Thompson 1990). In addition, the experiments must be sufficiently replicated to allow accurate measurement of the trait. The QTL mapping approach is difficult for QTLs with a very small effect.
- A different, although more laborious, approach is to use the advanced backcross QTL (ABQTL) method (Tanksley and Nelson 1996) to produce NILs.
- Once QTLs are identified a fine mapping population must be developed to locate the QTLs close to the marker.

3. Limitations to QTL studies for drought tolerance.

Using QTL analysis alone, it may be difficult to identify genes for MAS.

- With small mapping populations, a lot of uncertainty exists about the location of QTLs. It is hard to know whether two QTLs are really in the same place. Fine mapping is required, and this is time-consuming and expensive.
- In mapping populations, you collect data on both good and bad lines. This is different from a breeding program, in which you eliminate the bad lines as you advance generations. Including lines with high levels of genetic sterility or high disease susceptibility, for example, can confuse your results.
- Mapping studies require collecting yield data on a large number of lines across several environments. This is expensive and can be difficult to repeat. Many QTLs have been reported for suggested secondary traits (Table 2, Section 4.2), but there are not many reports of QTLs for yield under stress.

Sec. 4.2

Because of these limitations, additional techniques for gene discovery are now being applied to drought studies. These may result in candidate genes that can then be introgressed into suitable varieties through MAS.

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Authors' address: International Rice Research Institute, DAPO Box 7777, Metro Manila, Philippines.

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SECTION 5

Case studies of the application of approaches for breeding for drought tolerance

SECTION 5

CASE STUDY 1

Designing a national breeding program for developing drought-tolerant rainfed lowland varieties: the Thailand experience

Boonrat Jongdee

1. Our target environments (TPE)

The rainfed lowland is a major rice ecosystem in Thailand, with an area of approximately 5.7 million ha—more than 60% of the total rice land. The majority of the lowlands are in the northeast and north and are classified as shallow favorable and shallow drought-prone. Rainfall is bimodal and drought may develop early and late in the growing season. The early season drought occurs in most areas, affecting the time of transplanting of seedlings and the growth of direct-seeded rice. Late-season drought develops at the end of the monsoon season in most years in the northeast, particularly on the upper part of the toposequence of the paddy where water loss is greater from soil percolation and lateral water movement.

We used genotype by environment interactions (GEI) and cluster analysis of grain yield from multilocation trials to further define our target population of environments (TPE). However, groups of environments changed from year (Y) to year (large G × Y component of the GEI) and it was difficult to define G × L (location) groupings.

Recently, we changed the system of defining the TPE based on our work with farmers. We conducted a "Farmer Participatory Workshop for Production Improvement for Rainfed Lowland Rice in the North and Northeast" and from this identified the target domains based on hydrology. Three levels of toposequence are identified: upper, middle, and lower. The upper level can be defined as an unfavorable environment where drought can develop at any growth stage and the middle level as drought-prone where rainfall is variable and soils are light in texture. The lower level can be classified as less favorable because drought can develop in the early season followed by a sudden flood (Table 1).

Farmers' estimates of yield reduction because of late-season drought were 45–50% and 15–20% for the upper and middle levels, respectively. Early season drought is more frequent than late-season drought but yield loss is more severe in the latter. Thus, the target of our breeding program is cultivars with tolerance of intermittent and late-season drought. We use the different positions on the toposequence to represent differences in the severity of drought in our testing program.

2. Our breeding approach

In our past (traditional) breeding system, selection was mainly on-station and most lines were discarded based on visual selection and on the results from yield testing at a single location (i.e., local adaptation). Only a small number of lines (e.g., 50–70 lines) relative to the total

Table 1. Use of position on the toposequence to define the type of drought and the target population of environments (TPE) for the breeding program.

Position on toposequence	Type of drought	Yield loss in the TPE
Upper	Early, intermittent, and late	Late drought causes 45–50% yield loss
Middle (drought-prone)	Early and late	Late drought causes 15–20% yield loss
Middle (favorable)	Early	Minimal yield loss
Lower	Early and sudden flood	

(400–500 lines) produced from the crossing program were selected for subsequent interstation (wide adaptation) and on-farm performance. This selection system made it difficult to identify high-yielding lines at the farm level because of a large GEI for grain yield (Cooper et al 1999).

The breeding approach was changed to increase selection efficiency for the TPE and shorten the selection process. This change is based on the work and recommendations of Cooper et al (1999). The previous breeding program took 12–15 years; now the cycle is completed in 10–11 years. The selection cycle has three major phases: intrastation (local, on-station selection), interstation (across, on-station selection), and on-farm selection.

One of the recommendations was to replace the intrastation phase with early generation interstation yield testing of F_4 bulks to select for wide adaptation at an earlier stage of the selection process (Fig. 1). However, the F_4s are still segregating for flowering date and this causes some error in estimating grain yield. We now test large numbers of F_7/F_8 in interstation trials (multilocation) and we use the rapid generation advance (RGA) technique to develop the material for testing. We are now in the process of incorporating on-farm testing earlier in the selection process (see Fig. 2).

3. Our selection strategy

The different selection criteria used to develop cultivars for each of the TPE defined by the upper, middle, and lower terraces are shown in Table 2. Phenology, particularly flowering time, is the most important trait for avoiding late-season drought in each of the different domains. Flowering must occur before the standing water in the paddy disappears. Thus, we select three flowering groups—(1) early maturing: flowering around mid-September to the beginning of October, (2) intermediate maturing: flowering around mid-October, and (3) late maturing: flowering around late October—for the different domains of the toposequence.

We select for yield directly in the multisite selection program (described below) and we manipulate the water environment at a few sites to measure the drought-tolerance traits of flowering delay, spikelet sterility, and, increasingly, leaf water potential.

4. Water management to simulate late-season drought (at three test locations)

Managed environments are used to achieve repeatable drought screening.

Drought screening trials under water-managed conditions are conducted in the wet season, in which seeding is delayed by 2–3 weeks vis-à-vis the normal planting time. This increases the chance of developing a late-season drought. Also, the standing water is drained from the field around 2 weeks before flowering, when the earliest lines have reached the flag-leaf stage, to further induce drought stress during the targeted growth stage. If necessary, irrigation water is added to ensure free-standing water before flag-leaf exsertion.

In this trial, we measure grain yield, spikelet fertility, and flowering date, with a focus on spikelet fertility to complement direct selection for yield. We measure the percentage of fertile

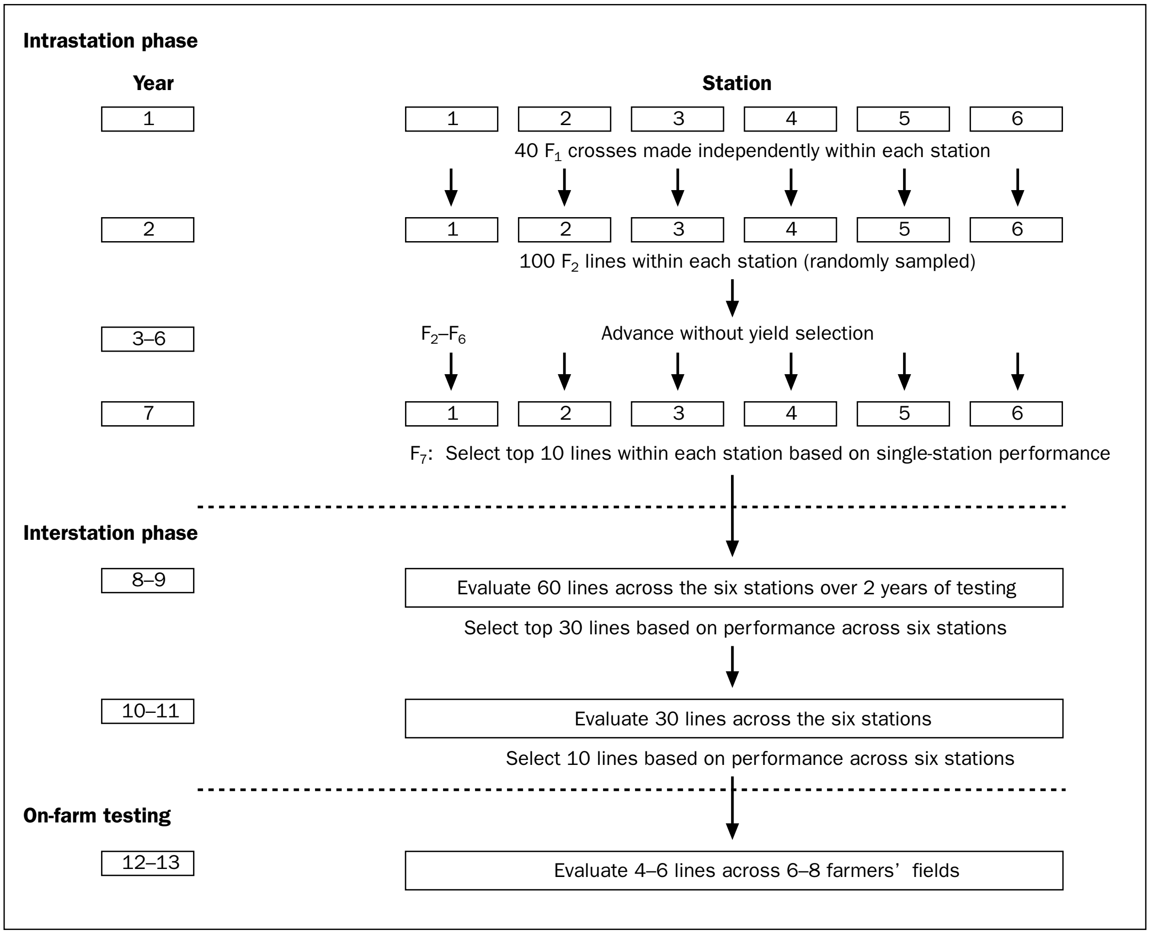

Fig. 1. Previous (traditional) breeding strategy.

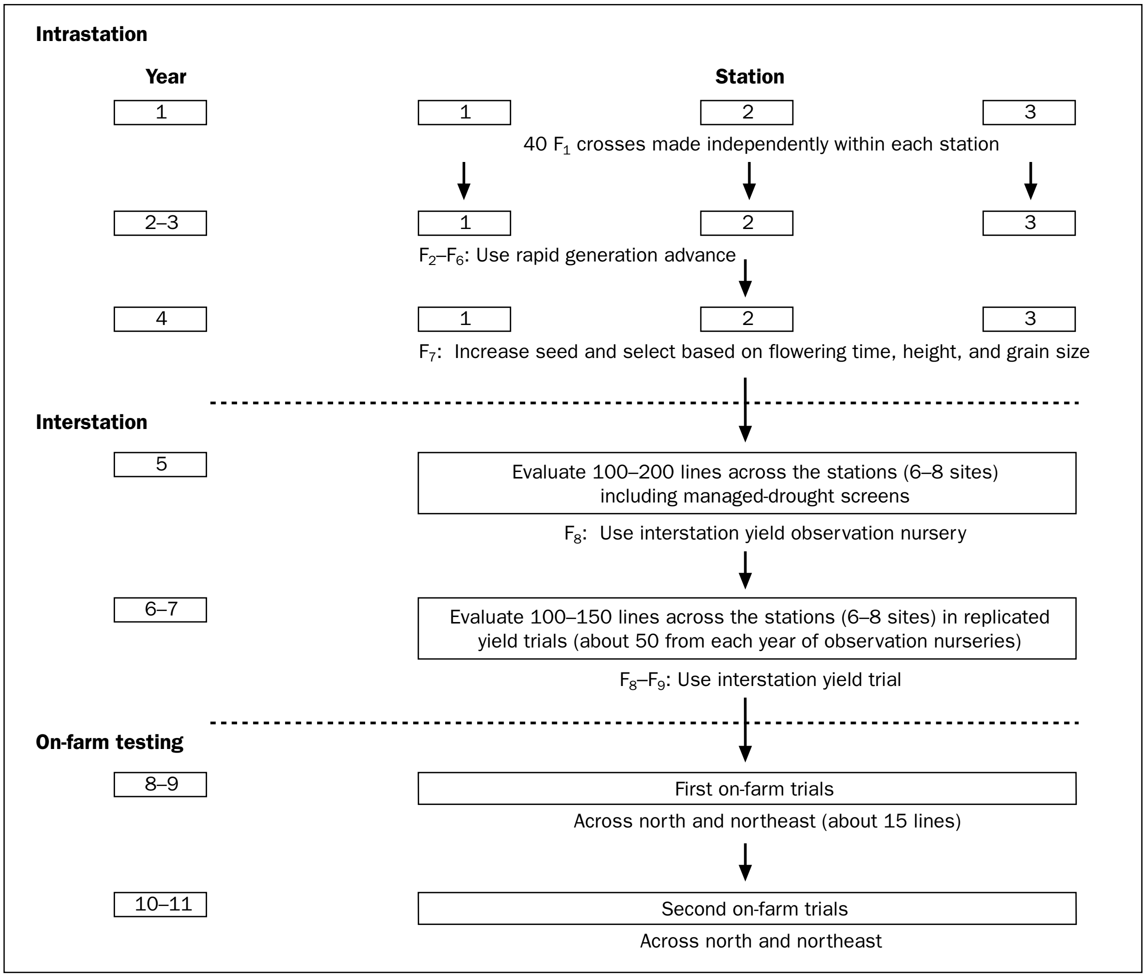

Fig. 2. A new selection strategy.

Table 2. Selection criteria to develop varieties for each target domain.

Target domain	Cultivar requirement	Selection strategy
Upper	Early maturity Low number of tillers Drought tolerance Less delay in flowering Low spikelet sterility Maintenance of LWP[a]	Select for yield under the test location
Middle (drought-prone)	Intermediate maturity Photoperiod sensitivity Intermediate height Drought tolerance Less delay in flowering Low spikelet sterility Maintenance of LWP	Select for yield under the test location
Middle (favorable)	High grain yield Intermediate height	Select for potential grain yield
Lower	Late maturity Photoperiod sensitivity Submergence tolerance	Select for yield under the test location

[a] LWP = leaf water potential.

spikelets from panicles that are harvested randomly in each line. Variation in flowering date among the test lines causes differences in the severity of drought stress and thus in spikelet fertility. To adjust for this effect, we compare spikelet fertility (and grain yield) among lines within the same maturity group.

We record the level of standing water in the paddy as well as the level of underground water below the soil surface as indicators of the type and severity of the drought. The observations are made once a week in all trials.

5. Selection and yield testing

Intrastation: crossing and rapid development of fixed lines for yield testing

A few research stations are involved in developing lines for yield testing (see Figs. 1 and 2). We select in the F_2 for characters with high heritability such as height, plant type, flowering time, and grain size (Fig. 2). Photoperiod-insensitive materials are advanced for two generations in the same year by growing them in the dry season. We use a dark room to induce flowering in photosensitive materials. No selection occurs during this process of advancement of materials.

Interstation trials: direct selection for yield and indirect selection for drought-tolerance traits at the station

Thirteen research stations across the north (five stations) and northeast (eight stations) are involved in the multilocation yield-testing program. The trials are conducted under two conditions of water availability: the water regime of the normal rainfed lowlands at 10 of the stations and a water regime that is manipulated to simulate late-season drought at three stations (two in the northeast and one in the north, see part 4 above). The objective of this selection is to evaluate families for grain yield under normal rainfed and late-season drought conditions. The F_7 lines are evaluated in two steps—an interstation observation trial and an interstation yield trial.

The interstation observation trial contains a large number of lines (200–300) grown in two replications and in plots of four rows, 2 m in length. In some cases, the lines are grouped by flowering time and form a separate trial, with each trial containing a set of check varieties that have been selected for their known response to different water environments. An Alpha-plus experimental design is employed. The data are analyzed using REML, SAS, and GENSTAT.

The selection in the interstation observation trial is based on grain yield under normal and manipulated late-season drought. The first analysis is of grain yield data from the normal water regimes from each of the 10 stations. The data are analyzed by station and also in a combined analysis across the stations. The lines are grouped based on the GEI analysis for yield into different patterns by cluster analysis. The groups of lines that perform well at most environmental sites are selected and the groups that have low grain yield in most environments are discarded.

Because variation occurs in flowering time among the test lines, the second analysis is conducted for lines within the selected groups. Individual lines are selected based on spikelet fertility (percentage) and grain yield under the manipulated late-season drought, bearing in mind the variation in flowering date. Lines with resistance to major diseases and insect pests and with appropriate grain quality are selected in this step as well.

The interstation yield trial is conducted across the same stations in the north and northeast using the same experimental design as that of the interstation observation trial but with three replications. The plot size is expanded to five rows, 5 m long. The lines can be grouped by flowering date if there are a large number of lines in each flowering group. This grouping facilitates trial management of the timing of fertilizer application and of bird control, and it allows for the adjustment of the effects of different flowering times (and therefore different levels of stress) on grain yield.

The selection of lines is based on grain yield under rainfed conditions and also under the manipulated late-season drought. The approach is the same as described for the observational trials. Again, there is selection for resistance to important insects and diseases and for grain quality characters.

Selection at the farm level

Our previous on-farm trials included only four to six lines with different flowering times and favored the selection of lines for shallow favorable conditions that are not representative of farmers' fields. More recently, Inthapanya et al (2000) suggested more rigorous testing in farmers' fields representative of their risk of drought and of soil fertility levels. We now plan to conduct two stages of on-farm trials:

- the first with a large number of lines in each of the three flowering groups of our target domain, in which 20 lines are grown with a small plot size (6–8 rows per plot), and
- the second with a small number of lines, with a large plot size (16 rows per plot). We also plan to invite farmers to participate in the selection of these materials.

6. Conclusions and next steps

We report here the changes that we made in our breeding program over the last few years. We do not know yet how successful we will be in developing better cultivars for farmers. However, we are confident that our changes are based on well-documented and well-reported research.

Now, we are focusing on selecting *parental material* based on more in-depth screening for sound physiological traits. We

- Use two techniques to induce drought—a line-source sprinkler and water-drainage before flowering as described earlier.

- Use the traits of leaf water potential, leaf death score, drought response index, flowering delay, and spikelet fertility to identify parents.
  - Measure leaf water potential at midday (1130 to 1500) on up to 60 plots per hour (1–3 leaves per measurement) per team of five people.
  - Record flowering time and grain yield under both well-watered and stress conditions and leaf death score and spikelet fertility under stress conditions.
- Select parents with increased drought tolerance for crossing with well-adapted and accepted commercial cultivars.

The progenies from these crosses will be used in the routine breeding program described above.

We are also conducting studies on the use of *molecular analysis* for drought tolerance. We aim to identify QTLs and develop a marker-assisted selection (MAS) scheme for traits related to yield performance under water deficit at the flowering stage such as leaf water potential, spikelet fertility, flowering delay, and drought response index. The doubled-haploid population derived from the cross between drought-tolerant cultivar CT9993 and susceptible cultivar IR62266 is being used. Some QTLs for the traits mentioned have been identified and potential markers of use in MAS are being identified.

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Notes

Author's address: Ubon Rice Research Center, Ubon Ratchathani 34000, Thailand.

SECTION
5
CASE STUDY 2

A regional breeding program to develop drought-tolerant rainfed lowland germplasm: IRRI's experience in South and Southeast Asia

Surapong Sarkarung

Regional trials can be a source of new parental lines for local breeding programs.

1. Introduction

The rainfed lowland rice ecosystem is heterogeneous and improved varieties must be developed at the local level. Also, end users (usually farmers) value the unique quality characteristics of their local materials. However, local breeding programs can benefit from the introduction and use of materials with broader adaptation to the major subecosystems of the rainfed lowlands. Thus, the breeding objective of the IRRI rainfed lowland program from 1991 to 2001 was to select traits that are effective for broad adaptation within a subecosystem. We anticipate that these materials can be further selected and used in breeding programs at the local level.

2. The target population of environments

There are two important target populations of environments (TPE) at the regional level: one in northeast Thailand (5 million ha) and the other in eastern India (10 million ha). Genotype by environment interaction (GEI) studies by Wade et al (1999) show that these two subecosystems have different environments and different genotype needs. IRRI therefore does breeding in these two subecosystems using a decentralized approach to population improvement to ensure that the selection environments are representative (Sarkarung 1995). IRRI provides diverse sets of segregating populations (F_2, F_3) and breeding lines to national agricultural research and extension systems for their use at the local level.

3. The breeding strategy

The cultivar requirements for the different TPE, based on the work of Singh and Dwivedi (1996) and Wade et al (1999), and the breeding strategy are shown in Table 1.

4. Developing populations and selecting parents

Success in breeding depends largely on the choice of parents. We have used for one parent a cultivar that is well adapted to the local environment and desired by end users. This local germplasm has shortcomings such as low yield ability, susceptibility to pests, and lodging susceptibility. We therefore add new sources of yield potential or resistance to biotic and abiotic stresses as the other parent.

Table 1. The target population of environments (TPE) and types of drought, cultivar requirements, putative traits under investigation, and the breeding strategy for the regional shuttle breeding program for the rainfed environments in Asia.

TPE and types of drought	Cultivar requirement based on proven traits	Putative traits under investigation	Breeding strategy
Eastern India			
Drought in the reproductive and vegetative stages	Medium maturity (125–140 d) Drought tolerance Resistance to bacterial leaf blight (BLB) and gall midge Intermediate height (120–130 cm) Photoperiod sensitive (flower 1 October) Yield potential of 3–5 t ha^{-1} Maintain local adaptation and quality	Root pulling Root penetration Osmotic adjustment Drought score	Direct selection for yield, both on-station and on-farm
Drought at seedling stage and submergence (flash flooding)	Medium to late maturity (120–150 d) Intermediate response to photoperiod Tolerance for early drought Withstand delayed planting Submergence tolerance Resistance to BLB and brown planthopper Maintain local adaptation and quality	Marker-assisted selection (MAS) for submergence genes[a]	Direct selection for yield, both on-station and on-farm Test for delayed planting Submergence screening Controlled drought screening
Submergence (deep water)	Late maturity (>140 d) Photoperiod sensitive (flower in November) Submergence (elongation) tolerance Resistance to waterlogging	MAS for submergence genes[a]	Direct selection for yield, both on-station and on-farm Controlled deepwater screening
Northeast Thailand			
Drought in the vegetative and reproductive stages	Medium duration (120–135 d) Weakly photoperiod sensitive (flower 15 September) Resistance to blast, BLB, BPH, and gall midge Intermediate height (115–135 cm) Target yield of 3–4 t ha^{-1} Good eating quality	Strong root system (root pulling) Stay-green Seedling vigor	Screening for maturity and pest traits On-farm evaluation of F_4 lines onward Controlled drought screening of F_5 (vegetative) and F_7 to F_8 (reproductive) Multilocation testing for yield
Drought in the vegetative stage	Medium duration (130–145 d) Photoperiod sensitive (flower 15 October) Resistance to blast, BLB, and BPH Intermediate height (110–130 cm) Resistance to lodging Target yield of 4–5 t ha^{-1} Good eating quality	Seedling vigor Deep roots (root pulling)	Same as above

[a] Based on the studies of Tochinda et al (2002).

The identification of parental materials and development of new populations are an ongoing process in any breeding program. We have developed populations based on Mahsuri, Safri 17, Rajshree Vaidehi, and Sabita for eastern India and KDML105 for northeast Thailand. We evaluated 3,000–4,000 cultivars/breeding lines from IRRI and CIAT and Asian traditional cultivars for their adaptation to poor soils, drought, and submergence. The selected materials were further tested for major diseases (blast, bacterial leaf blight), insects (brown planthopper, gall midge, green leafhopper), and grain quality and then used in the crossing program.

5. Evaluating and selecting segregating material for target environments

The populations developed for all of the TPE have been improved in a shuttle program based on the concept of testing and selection of each of the generations in a selection environment

Table 2. Overall breeding scheme for population improvement in the IRRI regional rainfed lowland breeding program.

Year	Generation/activity	Remarks
1	Test parental material Make initial cross	3,000–4,000 cultivars and breeding lines evaluated
2	F_1 planting Make 3-way and double crosses	
3	Screen F_1s of multiple crosses Evaluate and select F_2 (5,000 pedigree lines) in target environments and select for desirable traits	Select for highly heritable traits In Thailand, use MAS for grain quality
4	F_3 (500–700 lines), generation advance in dry season F_4 (500–700 lines), plant in target environments and screen for blast and BLB[a]	F_4 lines with similar maturity and plant height enter the on-farm evaluation in year 5
5	F_5 (100–200 lines), generation advance in dry season and screening for drought, BPH[b], and grain quality F_6 (100–130 lines), on-farm evaluation at 5 key sites and screening for blast and BLB	Begin imposed-drought screening
6–7	F_7 (30–50 lines), generation advance and screening for grain quality F_8 (25–30 lines), preliminary yield test at 5–7 key sites	
8–10	F_9–F_{10} (15–20 lines), advanced yield test at 10–15 key sites	
11–12	Elite lines (5–7) for demonstration and release	

[a] BLB = bacterial leaf blight. [b] BPH = brown planthopper.

that represents the TPE and then advancing in an "off-season" nursery in the region. Table 2 gives an example of the flow of material. The early generations (F_2 and F_3) are exposed to the normal conditions of the TPE, which often includes water-stress conditions. The following traits with high heritability (Jennings et al 1979) and those that can be visually identified are selected (rejected) in the early generation:

- Panicle density. Number of grains per panicle can be estimated from primary and secondary branches.
- Grain size.
- Grain type (visual). (Also, in Thailand, use MAS for aroma, amylose content, gel temperature, and gel consistency to select plants in the BC_2F_2 and BC_3F_2.)
- Tiller number. Total of 6–9 productive tillers.
- Plant height. Intermediate height (110–135) cm) is adequate to compete with weeds.
- Maturity period. The growth period from seeding to harvest should be made to fit the rainfall pattern of the target environments (eastern India, 130–150 days; northeast Thailand, 120–140 days).
- Susceptibility to diseases and insects (rejected).

Yield evaluation in each of the TPE begins at F_4 and includes evaluation in farmers' fields at some locations. In Thailand, the multilocation testing includes seven locations where the materials are exposed to drought stress, in either the vegetative or reproductive stage or both. The NARES in Thailand also have other testing locations, including farmers' fields, and some regional materials enter this testing network (see Section 5.1).

Sec. 5.1

The F_5 lines are screened for drought tolerance in a special trial in the dry season using sprinkler irrigation. The main purpose is to identify genotypes that can withstand drought stress in the vegetative stage. The protocol for the controlled drought screening involves

- Test materials: breeding materials, traditional cultivars, and released varieties grouped by known maturities.
- Check varieties: local checks (resistant—NSG19, intermediate—KDML105, and susceptible—IR20, Mahsuri).
- Plot size: four rows 1.0 m long, spacing 0.15×0.15 m^2, 4 replications.
- Planting method: dry seeding with one plant $hill^{-1}$ after emergence.
- Irrigation pipes: irrigation water is needed before drought is imposed at 45 days after emergence and again to relieve the stress after flowering (35 days later).

Some 1,000–3,000 F_5 lines are screened in each planting. The measurements that are routinely undertaken in the dry-season drought screening are

- *Drought score*. This is taken at weekly intervals when the susceptible checks display drought symptoms, which include leaf-tip drying, yellowing, and stunting.
- *Relative water content*. Measurements are taken after midnight (from 0100 to 0600) and at midday (1130–1430) at the maximum tillering stage, 2–3 times at weekly intervals.
- *Canopy temperature*. This is measured after the water is cut off at about 40–45 days after seeding. The canopy temperature is taken under intense sunlight from 1100 to 1430.
- *Recovery score*. This is measured 1 week after rewatering by visually rating the emergence of green leaves.

New varieties based on selection for yield and drought tolerance traits are being evaluated by farmers.

6. Progress and evaluation

Materials from this program are now being used and incorporated by the NARES in their breeding programs (objective of the regional program). Some early examples of the use of these materials can be seen in the following examples.

Eastern India

The rice-breeding populations in eastern India have been improved mainly for the more highly heritable traits such as submergence tolerance. When the materials were evaluated on-farm with a set of diverse breeding lines (F_4 onward and 15–20 lines per site), many lines were selected by farmers to suit their needs (grain type, straw quality, maturity period, etc.). The most promising lines have been nominated to the national testing program for formal release.

Northeast Thailand

The major breeding objective for northeast Thailand and other countries in Southeast Asia is premium grain quality. Several lines have been selected that combine high quality and improved response to drought (Table 3) and these are in the final process of on-farm testing.

Table 3. Elite lines that combine high grain yield and field drought tolerance in farmers' fields in northeast Thailand.

Cultivars/advanced lines	Parents/donors	Drought response	Remarks
IR68796-27-3-B-5-2	KDML105, OS6, Kurkaruppan, Latisail	High recovery	On-farm testing, good eating quality, possible for release
IR69515-27-KKN-1UBN-1-1-1	KDML105, Latisail, Dhalputtia, Patnai, Nahng Mon	Tolerance at vegetative stage	On-farm testing, blast resistance
IR68835-28-2-B-1-4-B	KDML105, Hawm Dong, Latisail	Tolerance at vegetative stage	On-farm testing

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Author's address: 60/625 Krisdanakorn 25, Prachuruamjai Road, Minburi, Bangkok 10510, Thailand.

SECTION
5
CASE STUDY 3

Integrating selection for drought tolerance into a breeding program: the Brazilian experience

Beatriz da Silveira Pinheiro

1. Our target environments (TPE)

Brazilian savannas have a well-defined rainy season, starting in October and ending in April. During this period, total rainfall ranges from 1,200 to 1,500 mm, with monthly averages sometimes higher than 200 mm. In spite of this abundance, rain distribution may be irregular and dry spells can occur, most frequently during January and February, when the upland rice crop, sown at the onset of the rainy season, undergoes reproductive development.

In the early years of the Cerrado (Brazilian savanna) opening, upland rice was the most attractive pioneer crop because of its rusticity and tolerance of soil acidity and low fertility. In the 1970s and ’80s, a large area was deforested for agricultural activities and rice attained its peak of 4.5 million ha under cultivation in 1987-88. During this period, while area and production increased, upland rice yield was low and constant at around 1.2 t ha^{-1}. Yields remained low because of the combined effect of dry spells and low adoption of recommended technology. Thus, our *initial TPE* was for the drought-prone uplands with low fertility, with a focus on developing varieties with tolerance of midseason reproductive drought, relying on the japonica group as the major source of germplasm.

The TPE can change when farming practices change.

After the studies of Pinheiro et al (1985) and Steinmetz et al (1985), the breeding strategy was expanded to include selection for yield potential (modern plant type) to obtain genotypes to be grown under supplementary irrigation and in favorable microregions with desirable rainfall distribution. Initially, this new TPE, aimed at favorable upland conditions, required only a small share of human and financial resources and used predominantly indica germplasm. With time, the decline in savanna frontier land and concomitant migration from the southeast to northwest, that is, from a riskier toward a less risky environment (Steinmetz et al 1988), resulted in a decline in upland rice area (2.4 million ha in 2001) associated with increased average yield (1.9 t ha^{-1}). Thus, the *target domain has changed* to more favorable conditions (Photo 17) and the breeding priorities have shifted to include yield potential and improvement of grain quality, and to rely more and more on japonica by indica crosses. Plant architecture and grain appearance are now important requisites for variety release for both favorable and unfavorable climatic conditions, so the distinction between the two former upland breeding programs, aimed at the different TPE, has disappeared.

2. Our breeding approach for drought-prone areas

The dramatic shift of the crop to more favorable locations and the higher use of technology by farmers, which help minimize risk, led to a decrease in the priority for drought tolerance in the upland rice breeding program. Now, because of the move in plant type from tropical japonica to japonica × indica derivatives, yield potential has increased from 4.5 to 6 t ha^{-1} and average yield has doubled. Accordingly, the support program on drought-tolerance evaluation has been reduced and drought-stress tolerance, considered previously as a major research priority in the national upland rice breeding program, now plays a secondary role to improvement of yield under favorable environments. However, here we describe the initial approach used by the upland rice breeding program of EMBRAPA Rice and Beans (formerly the National Rice and Beans Research Center, CNPAF) to develop varieties for the TPE of unfavorable savanna areas with midseason drought. We discuss the successes as well as difficulties and limitations of the program.

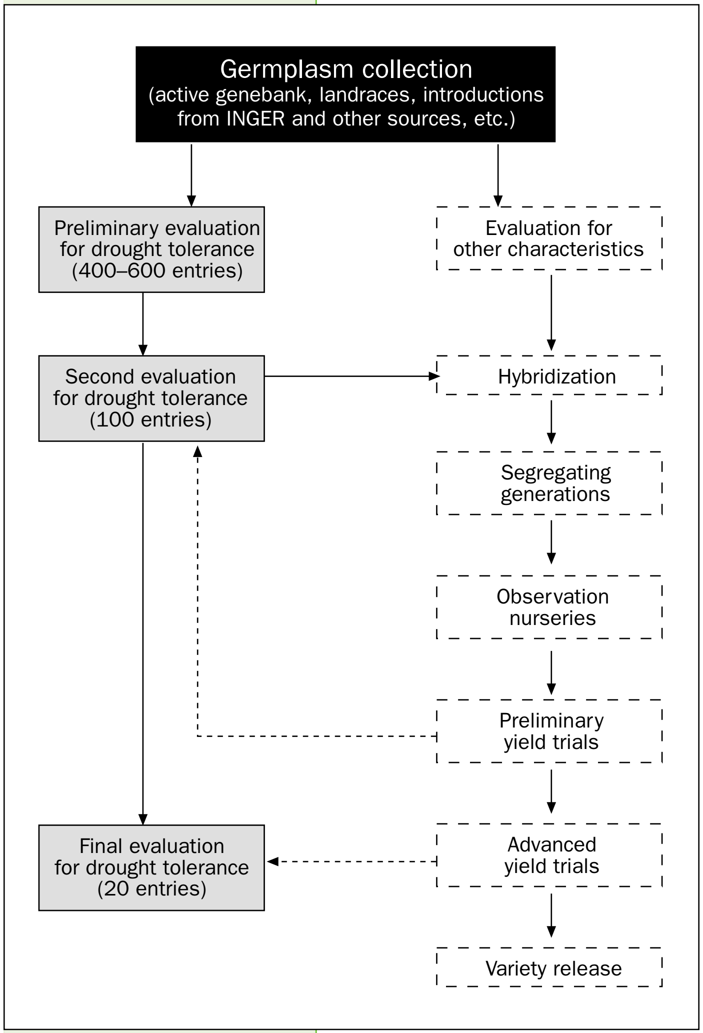

Fig. 1. Schematic representation of germplasm flux in the drought-tolerance evaluation program and its relationship with the upland rice breeding program aimed at unfavorable conditions.

From the start, we found that the unpredictable occurrence of drought and its timing with the critical stages of plant development made selection among segregating materials in a conventional breeding program inefficient. Therefore, we focused on (1) the careful evaluation of potential progenitors using drought-tolerant selections for crossing to elite germplasm and (2) testing advanced (fixed) breeding lines under controlled drought conditions. These two drought selection activities were conducted as support to the routine breeding program and involved a strong partnership between plant physiologists and breeders in three classes of experiments, designated as "preliminary evaluation," "second evaluation," and "final evaluation" (left side of Fig. 1). Such trials, described in more detail below, were conducted at CNPAF's headquarters in Goiânia, Goiás.

Figure 1 shows the overall approach of this breeding and evaluation work. The program began by focusing on selecting for drought-tolerant materials to be used in crossing. The genotypes used as parents were predominantly of tropical japonica extraction, from both national and African origin. They were screened in the preliminary and second evaluation to identify those for use in the crossing program. The final evaluation for drought, in the original strategy, contained the best selections from the second evaluation and the most promising lines from the second year of testing in the advanced yield trials (right side of Fig. 1). The methodology of this drought evaluation trial, which is still part of the breeding program, is described in detail later.

3. Identifying the parents

The *preliminary evaluation* trial for drought tolerance (see Fig. 1) comprised 400 to 600 entries each year, including local varieties, some regionally collected ones, and elite germplasm from national and international programs. The experiments were planted late in the season, in January (recommended sowing time is 15 October to 15 November), to improve the probability of drought during reproductive development. The plots were kept well watered during the vegetative stage until a significant proportion of the entries were in the reproductive stage, when irrigation was discontinued to induce water stress. If stress occurred, entries were evaluated using the IRRI standard drought-tolerance visual scale (Chang et al 1974, Loresto et al 1976), with scores from 1 to 9. Entries that ranked equal to or better than the commercial varieties IAC 25 and IAC 47 (respectively, checks for early and late maturity) in this trial, as well as the outstanding entries from the preliminary yield trial, were selected for the second evaluation trial (see Fig. 1).

In this *second evaluation*, the entries were grouped into early (less than 80 days from sowing to flowering), medium (80–90 days), and late (more than 90 days) maturity classes to allow for a comparison among genotypes with similar maturity. The sowing date was staggered by maturity (i.e., the late group was sown 10 days before the medium group and 20 days before the early group). The field arrangement was similar to that of the preliminary evaluation, but the number of replications increased to at least three and a fully irrigated treatment was included. The protocol to induce water stress (in the drought treatment) was the same as for the preliminary evaluation. Again, no yield data were collected from the plots and drought evaluation was based on IRRI's visual scale for drought tolerance at the reproductive stage.

The topmost entries were recommended as parents for hybridization with elite varieties and advanced breeding lines.

4. Yield testing

The segregating lines or populations derived from these crosses of drought-tolerant with elite materials were evaluated for yield as part of the normal plant-breeding program. The selection also considered visual characters related to stress escape and avoidance such as a short growth cycle, moderate tillering ability, and moderate leaf area, as well as agronomic characters and reaction to biotic stresses. If drought stress occurred in the routine yield testing, individual lines were discarded on the basis of leaf rolling, panicle exsertion, and spikelet fertility.

The fixed lines were then evaluated for yield in three classes of experiments: observational nurseries, preliminary yield trials, and advanced yield trials (right side of Fig. 1). Selections from the advanced yield trials were then included in the final drought-tolerance trials (see left side of Fig. 1) before recommendations were made for varietal release. Genotypes with high yield potential or desirable grain traits, as identified in the yield trials, could be discarded in favor of more drought-tolerant genotypes, identified in the drought evaluation trials.

At first, all of these yield trials were conducted only at CNPAF's headquarters in Goiânia, Goiás. However, in 1983, the Regional Commission for Testing and Recommendation of Rice Varieties was established with 15 public research institutions and 18 sites to conduct preliminary and advanced yield trials of breeding lines from three institutions for the savanna region. In this network, trials are conducted during the normal growing season, without controlled irrigation.

In the original strategy, the final evaluation for drought contained the best selections from the second evaluation and the most promising lines from the second year of testing in the advanced yield trials (right side of Fig. 1). Since the main objective was to use the information to decide on variety release, this trial compared yield under irrigated and drought treatments of entries whose flowering period matched through staggered sowing. Desirable breeding lines and varieties for release were those that had relatively high yield under drought relative to that under irrigation and an absolute yield under drought equal to or greater than that of the check variety of a similar maturity class. The methodology of this drought evaluation trial is still part of the present breeding program.

Promising lines are evaluated under managed drought.

5. Managing the final drought evaluation trials

The *final drought evaluation trials* are planted late with multiple sowings to collect data on drought response despite the significant year-to-year variation at the onset of the dry season. This trial can accommodate 18 to 20 advanced lines and two to four drought-tolerant checks of different growth duration. Ideally, the experimental design is a randomized complete block with split plots, composed of water treatments (stress and sprinkler-irrigated control) and subtreatments (tested lines), with at least four replications. To prevent interference of one water treatment upon the other, there is a safe distance between irrigated and stressed plots, which means that water treatments within the same block need some spatial separation. For this reason, this fully randomized experimental design is not always used in our conditions. In most years, depending on area availability, the irrigated control is conducted as a complementary experiment in the same experimental area.

The entries tested are arranged in a minimum of four rows, 5 to 6 m long, spaced 0.40 m apart. The latest-maturing materials are sown from 2 to 6 weeks in advance in relation to the earliest ones, depending on the relative differences in their growth cycle duration, to allow for a reasonable synchronization of reproductive development. In the case of multiple sowing dates, the sequential sowings of the same entry are spaced 1 week apart.

Sowing dates are not considered as part of the experimental treatments in the analysis—rather, one sowing date for each entry (the one that best matches the flowering time) is used to evaluate drought tolerance. Entries whose date of flowering falls within a deviation of more than 5 days from the average date of flowering are discarded. Depending on the homogeneity of the area, it is preferable to reduce the number of sowings and maintain the desired number of rows per plot. It is also highly recommended to have three extra rows surrounding the entire experiment to minimize border effects and provide some protection against insects and diseases. Since these experiments are grown out of season, they are especially prone to various pests, including birds. Sorghum rows or extra plots may act as attractants to birds and provide some degree of control.

We endeavor to provide a uniform drought stress by

- *Rotation of the site.* Upland rice yields decrease noticeably after the second consecutive sowing in the same area. To avoid this problem, a homogeneous experimental area of approximately 2 ha was divided into two modules of 1 ha each. The fields are rotated with soybean or maize, followed by rice, and the area is sown to pasture for 2 years after the rice. In this way, the field is sown to rice only once every 4 years.
- *Uniform soil preparation.* The occurrence of hard soil pans, superficial soil compaction, and any soil physical or chemical discontinuity must be avoided to minimize the already high spatial variation when drought stress is applied. Soil preparation begins at the onset of the rainy season, in early October, with deep plowing, using a moldboard plow, to incorporate previous crop residues, followed by repairing of the levees. The area is then left undisturbed until planting of the experiment. Depending on weed contamination, herbicide application may be necessary, followed 1 week later by a light harrow to level the soil and incorporate any vegetation residue.
- *Uniform distribution of irrigation.* Water is applied to both the irrigated treatment and during the vegetative stage of the drought treatment, whenever rainfall is below pan evaporation for a period of 4 consecutive days. The irrigation sprinklers are carefully placed to ensure uniformity. The distribution is checked by installing cans at various distances from the sprinklers, just above the plant canopy. It is desirable to saturate the soil by applying excess water at the last irrigation before beginning the drought treat-

Uniform conditions in the drought nursery are essential.

ments. Soil samples are taken the day after the last irrigation and then at weekly intervals to determine soil moisture content.

- *Beginning of the stress.* The decision on when to induce stress has implications for the level of stress imposed and the timing of stress may be constrained by weather conditions. At our experimental site, rains decline substantially in April, but occasional showers can disturb the evaluation protocol. In contrast, almost no rain occurs in May, but low temperatures may occur. This temperature hazard, although occurring less frequently (usually one in four years) than the April showers, may cause the loss of data in some years.
- *Monitor the plant water status.* In addition to monitoring soil water content, we measure leaf water potential to monitor the average plant stress level (see Fig. 2). The combined measurements of leaf water potential and soil water content help determine when to terminate the stress for the targeted yield reduction. In our experiments, leaf water potential is measured twice a week, from 1300 to 1500. We usually monitor the two checks plus a few entries, randomly chosen. We use a pressure chamber and measure a maximum of six genotypes per block with four leaf samples per genotype, measuring two samples at a time. We usually rewater before water potential values become lower than –2.0 MPa to avoid excess damage to the plants and to achieve our target yield.

  At our site, under the prevailing climatic conditions of the dry season, and starting the stress imposition under full soil water saturation, it normally takes from 15 to 20 days to attain an adequate level of stress. An average vapor pressure deficit of 18 to 22 millibars at 1500, kept for 18 to 22 days, is capable of inducing a 30% yield loss in the resistant check.

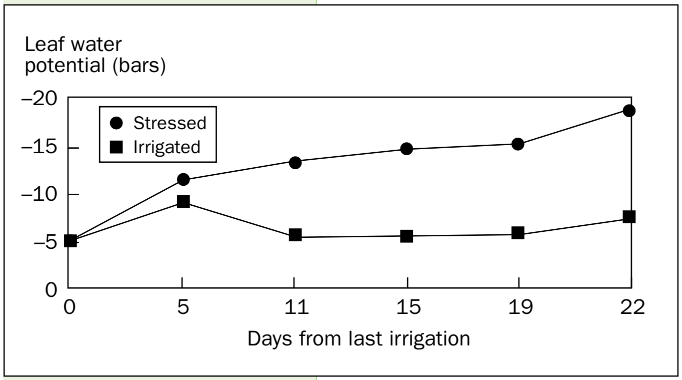

Fig. 2. Measuring the water status of plants under the drought stress and in irrigated treatments.

Compare the performance of plants that flower at the same time and under the same drought conditions.

- *Comparing genotypes at the same plant development stage.* Adjustments of sowing dates allow for a certain degree of synchronization among the reproductive stages of most lines. However, even small differences in phenological development may represent a significant difference in drought response. Plant size also influences plant response to drought. Consequently, comparing genotypes of different growth cycles would normally favor the early ones that develop a smaller leaf area in relation to the late ones. For this reason, it is best to confine comparisons among those entries of the same maturity group and it is necessary to include two or more checks of different growth duration. We now use Guaraní as an early check and Rio Paranaíba as a late check, replacing IAC 25, IAC 165, and IAC 47, now highly susceptible to blast.

It is important to remember that drought takes place some time after the rains subside or irrigation is discontinued. At our site, under the prevailing evaporative demand of the dry season, it normally takes from 5 to 7 days for a stressed plot to be differentiated from the irrigated control. So, when selecting the desired target stage for drought induction, care must be taken to make sure that induction begins in time to allow for adequate drought pressure during the targeted period. We choose flowering and early grain filling as the critical period and normally provide the last irrigation when around 10% of the tillers are at booting.

6. Using direct and indirect evaluation criteria for drought tolerance

Use direct and indirect measures of drought tolerance to evaluate lines.

We found it very difficult to attribute a drought-tolerance score based strictly on visual criteria during the stress. However, in the early years of the program, when some thousands of entries were being evaluated, we relied on the visual score as the only possible alternative and it was very helpful.

To evaluate the response of promising advanced lines for variety release, we use yield under stress and the relative yield loss in relation to the irrigated control (drought index) as the main criteria. Such experiments, however, require a large and uniform experimental area in addition to good crop and water management to assure adequate plant growth and uniformity.

We use the *indirect measures of drought tolerance of*

- delay in flowering,
- leaf rolling and leaf drying,
- panicle exsertion and panicle size, and, especially,
- spikelet fertility.

Box 1. **Measuring spikelet fertility.**

Visual scoring

Differences among entries are detected and translated into numerical scores. We rate spikelet fertility from 1 to 9, using the scale below.

Score	Percent spikelet fertility
1	Higher than 90%
2	80–90%
3	70–80%
4	60–70%
5	50–60%
6	40–50%
7	30–40%
8	20–30%
9	Lower than 20%

Measure the percentage of unfilled grains

Sample a reasonable number of panicles per plot, separate the empty and filled grains (here both the complete and partially filled caryopsis are included), and count only the empty ones. Then, determine the number of filled grains in an adequate subsample of the total sample and weigh the whole sample. The number of filled grains is then determined by simple calculation.

In our experience, spikelet fertility is the most useful visual indicator of the response of upland rice during the reproductive stage and, whenever it is more easily assessed than yield (see Box 1 for how to measure spikelet fertility), we see no restriction to using it instead. It also has the advantage of not being influenced by factors other than drought in the grain-filling period.

The correlation of these indirect traits with grain yield under stress is shown in Table 1. Note that

- yield under drought is correlated (r = 0.6) with yield potential,
- spikelet fertility is highly correlated with yield under drought conditions,
- leaf rolling and leaf water potential have a low correlation (nonsignificant) with yield under drought, and
- there is a significant correlation between yield under stress and the number of days from stress imposition to flowering.

The drought response of the tested entries still has some confounding effect with growth stage even though we staggered the planting to match the reproductive development of the tested entries.

7. Some examples of the response of different rice lines under drought

Table 2 shows the performance of 18 varieties—ten from CNPAF (CNA lines), two from CIRAD (IRAT 216 and IRAT 335), two from the Agronomic Institute of Campinas (IAC

Table 1. Simple correlation coefficients between traits measured in the irrigated and water-stressed trial.

Trial	Trait								
	Stressed yield[a]	Straw yield	Stress timing[b]	Number of panicles	Number of spikelets panicle^{-1}	Spikelet fertility	Weight of 100 grains	Leaf rolling	Leaf water potential
Irrigated yield	0.60**	0.328 ns	–	–0.067 ns	0.143 ns	0.339 ns	0.427 ns	–0.257 ns	–0.176 ns
Stressed yield	–	–0.353 ns	–0.541**	–0.226 ns	0.056 ns	0.842**	0.481*	–0.317 ns	0.174 ns

[a] * and ** = significant at 5% and 1% level, respectively. [b] Number of days from beginning of stress to date of 50% flowering in the stressed trial. ns = nonsignificant.

Table 2. Results of a final evaluation trial involving 14 upland advanced lines originating from the regional yield trial of the Brazilian breeding network. Numbers followed by the same letter are not significantly different at the 5% probability level.

Entry	Yield under drought stress (g m^{-2})	Yield loss[a] (%)	Flowering date[b]	Stress timing[c]	Straw biomass (g m^{-2})	Spikelet fertility (%)	Leaf rolling score	Midday leaf water potential (MPa)
IAC 84-198	235.3 a	19.8 cd	68.7	6.7 fg	304.1 de	71.2 ab	4.3 ab	–1.75 a
Guaraní	233.6 a	20.2 cd	69.3	7.3 fg	344.7 cde	81.1 a	4.3 ab	–1.88 a
CNA 6710	221.7 ab	25.8 bcd	73.0	9.0 def	350.9 cde	63.0 abc	4.3 ab	–1.89 a
CNA 6891	218.1 ab	18.9 cd	84.7	7.7 efg	525.7 abcde	55.7 bcd	4.2 ab	–1.88 a
IRAT 216	211.4 abc	35.0 abcd	93.0	9.0 def	622.8 ab	51.5 bcde	4.3 ab	–1.84 a
IAC 1176	203.8 abc	28.6 bcd	65.7	5.7 g	380.5 abcd	63.8 abc	4.5 ab	–1.87 a
R. Paranaíba	190.2 abcd	38.7 abcd	98.3	14.3 a	516.4 abcde	56.5 bcd	4.5 ab	–1.68 a
CNA 4140	188.4 abcd	36.5 abcd	94.7	10.7 cd	497.8 abcde	50.7 bcde	4.2 ab	–1.67 a
IAC 165	182.8 abcde	9.8 a	69.7	7.7 efg,	338.2 cde	67.9 ab	4.5 ab	–1.91 a
IAC 1175	131.8 bcdef	47.4 abcd	71.3	9.3 def	386.5 bcde	46.5 bcdef	4.8 a	–1.80 a
IAC 47	123.8 cdef	52.9 abc	98.0	14.0 ab	480.6 bcde	40.8 cdefg	4.5 ab	–1.86 a
CNA 6881	97.3 def	56.8 abc	94.0	7.7 efg	632.0 ab	20.5 g	3.8 b	–1.80 a
CNA 6187	92.0 ef	64.6 ab	96.7	12.7 abc	574.9 abc	35.0 defg	4.5 ab	–1.73 a
CNA 7101	90.2 ef	55.8 abc	88.0	11.0 cd	521.7 abcde	39.3 cdefg	4.7 a	–1.77 a
CNA 7127	87.1 f	66.1 ab	87.0	10.0 cde	631.1 ab	23.7 fg	4.2 ab	–1.79 a
CNA 7141	70.8 f	65.2 ab	95.0	11.0 cd	548.9 abcd	34.1 defg	4.8 a	–1.88 a
IRAT 335	64.3 f	54.9 abc	72.0	10.0 cde	276.2 e	24.3 fg	4.5 ab	–1.79 a
CNA 7066	55.0 f	76.4 a	95.5	11.3 bcd	753.3 a	26.9 efg	4.8 a	–1.80 a
F value	13.8**	6.0**		20.1 **	7.1 **	13.9**	3.4 **	1.1 ns
CV (%)	20.3	32.4		9.3	18.1	11.9	7.1	6.6

[a] Percentage yield reduction of the stressed plots in relation to the irrigated plots. [b] Number of days from sowing to 50% flowering. [c] Number of days from beginning of stress to date of 50% flowering in the stressed trial.

lines), two resistant checks (Guaraní and Rio Paranaíba, of short and long growth duration), and traditional upland varieties IAC 47 and IAC 165—evaluated in the managed-drought experiment of the regional advanced yield trials of the national breeding network coordinated by EMBRAPA.

The 18 entries were separated into three groups according to growth duration and sowing took place on five dates, spaced 1 week apart, with three sowings per group, beginning with the latest group and ending with the earliest one. Drought stress began on 5 May and was relieved on 27 May. Most of the entries in the irrigated plots flowered (50% flowering) from 12 to 19 May. On the last day of stress imposition, water potential ranged from –1.6 to –2.0 MPa and leaf rolling attained values of 4 to 5 in the stressed plots. Note that

- The drought-tolerant checks Guaraní and Rio Paranaíba had a higher yield and spikelet fertility than most of the entries of corresponding growth duration.
- The yield loss of Rio Paranaíba, the medium-duration check, was more than that of the short-duration check, Guaraní. This effect of maturity is normally observed in this kind of experiment, that is, the response to drought has to be evaluated within the same

maturity group; otherwise, long-growth-duration genotypes are seldom classified as tolerant.

- Independent of the growth cycle duration, we consider entries that lose less than 30% yield in relation to the irrigated control as tolerant, from 30% to 50% as moderately tolerant, from 51% to 80% as moderately susceptible (MS), and from 80% onward as susceptible (S).
- The data from the managed-drought trials were used to recommend the release of CNA 4140, IRAT 216, and CNA 6187 (Rio Paraguai, Rio Verde, and Carajás, respectively) and to discard CNA 7066, although it has excellent grain appearance.
- The performance has to be tested for at least two years to establish drought response with reliability.

8. Lessons learned from the drought evaluation program

Stress timing is critical for making progress.

The main problem we face is assuring adequate levels of drought stress at the target growth stage. Our experimental farm is in a low to medium climatic risk zone for the normal cultivation season. Our strategy of delaying planting, although increasing the chances of inducing drought at the desired plant growth stages, is not completely rain-proof. Moreover, in some years, low night temperatures may induce spikelet sterility, thus masking results and contributing to failure as well. Delayed sowing may also cause some undesirable effects on plant size and growth duration, besides increasing the incidence of blast. Nevertheless, the strategy worked well in a reasonable number of years.

Because of their adequate performance under drought conditions, traditional upland genotypes (landraces) collected regionally during the 1970s were used extensively as progenitors in the early period of CNPAF's upland breeding program. However, the majority of the derived pure lines were subsequently discarded because of their high susceptibility to blast. In the same period, several African genotypes were also used as parents and the crosses with 63-83 and improved Brazilian varieties gave origin to the widely used cultivars Guaraní (IAC 25 × 63-83) and Rio Paranaíba (IAC 47 × 63-83), both released in 1986 (Table 3).

The strategy of confining crosses to progenitors of the japonica group showing adequate drought tolerance has proved useful. The varieties derived from such crosses possess a higher

High-yielding drought-tolerant varieties can be developed by selecting for both yield and drought tolerance.

Table 3. Year of release, progenitor group, growth cycle duration, plant and grain type, and response to drought of upland rice releases from EMBRAPA Rice and Beans to the savanna region of Brazil.

Cultivar	Year of release	Progenitor group	Growth duration	Plant type	Grain type	Drought tolerance[a]
Cuiabana	1985	Japonica, indica	Medium	Traditional	Long bold	MS
Guaraní	1986	Japonica	Early	Traditional	Long bold	T
Rio Paranaíba	1986	Japonica	Medium	Traditional	Long bold	MT
Araguaia	1986	Japonica, indica	Medium	Traditional	Long bold	MS
C. América	1987	Japonica	Early	Traditional	Long bold	MT
Rio Paraguai	1992	Japonica	Medium	Traditional	Long bold	MT
Rio Verde	1992	Japonica	Medium	Traditional	Long bold	MT
Progresso	1993	Japonica, indica	Medium	Modern	Long slender	MT
Caiapó	1994	Japonica, indica	Medium	Traditional	Long	MT
Carajás	1994	Japonica	Early	Traditional	Long bold	T
Maravilha	1996	Japonica, indica	Medium	Modern	Long slender	S
Primavera	1996	Japonica, indica	Early	Modern	Long slender	MS
Canastra	1996	Japonica, indica	Medium	Modern	Long slender	MT
Confiança	1996	Japonica, indica	Medium	Modern	Long slender	MS
Carisma	2000	Japonica, indica	Early	Modern	Long slender	MS
Bonança	2001	Japonica, indica	Medium	Modern	Medium slender	MS

[a] T = tolerant, MT = moderately tolerant, MS = moderately susceptible, S = susceptible.

degree of drought tolerance than those involving indica sources of blast resistance, such as Cuiabana and Araguaia, released in the same period. Moreover, releases after 1994, such as Maravilha and Primavera, developed after some changes were made in the breeding program strategy, show less drought tolerance.

Recovering the original level of drought tolerance in the new releases is feasible. The variety Canastra (Table 3) as well as some new advanced lines recently tested (data not shown), all japonica by indica derivatives, have shown the same level of drought tolerance as their original japonica progenitors. In this new generation of crosses, a more adequate balance among plant type, grain quality, and drought tolerance has been achieved.

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Notes

Author's address: Embrapa Arroz e Feijão, Caixa Postal 179, 75.375-000 Santo Antônio de Goiás, Goiás, Brazil.

SECTION
5
CASE STUDY 4

Using marker-aided selection for a specific drought-tolerance trait

B. Courtois and R. Lafitte

Traditional upland rice varieties, shaped by generations of selection pressure under aerobic conditions, are among the most drought-tolerant and they have a deep and thick root system (Mambani and Lal 1983). An important objective of upland rice breeders is to keep this drought-tolerance trait while improving yield potential. The following describes the use of marker-assisted selection (MAS) for root traits. The work is also reported elsewhere (Courtois et al 2000).

1. Defining the breeding objectives—interest in root depth for the upland target population of environments

Sec. 2

An agroecological classification of the upland environments of Asia based on rainfall, drought occurrence and severity, and other nonclimatic factors is presented in Section 2, Table 2, of this manual. Three subecosystems are identified. In each of them, although drought patterns differ, there is a high risk of short periods of water deficit lasting 1 or 2 weeks. Our breeding objective is to avoid the drought stress by a deep root system that can take up water at depth in the soil profile. (The approach is successful only if there is water in the soil profile.)

To improve (or maintain) root depth, breeders need a reliable large-scale screening technique, an understanding of the trait variability in the germplasm dealt with, and information on its genetic control.

2. Our breeding approach

2.1 Screening technique

The root system has been called “the hidden half” because it is difficult to measure. Most direct screening techniques are tedious and cumbersome. Two situations can be considered: to grow plants under field conditions with replicated samplings of part of the root system with a root sampler or to grow plants in pots under greenhouse conditions (or in hydroponics and aeroponics, Price et al 1997) with access to the whole root system.

The plasticity of the root system is well documented. Under field conditions, many nongenetic factors can affect the expression of genetic potential. Soil variability, caused by differences in texture, aeration, mechanical impedance, water and nutrient availability, or chemical gradients, can be dealt with by multiplying the samplings but the effort can be out of proportion with the resources of many breeding programs. In addition to soil variability, some biological factors affecting root growth, such as pathogenic nematodes, are very difficult to control. Work at IRRI showed that the broad-sense heritability of root depth under field conditions was very low (Courtois et al, unpublished results) with, in some cases, the uncontrolled variance higher than that for the genotypic variance. Because of these limitations, we attempted to develop an indirect field technique based on injection of a herbicide, metribuzine, at depth using a veterinary syringe with long needles. However, this selection technique was not reliable or conclusive (Trebuil et al 1996).

For all these reasons, we evaluated our material in pots in the greenhouse. Although this selection environment may not fully represent the conditions in the target population of environments (TPE), it ensured better control of the soil properties—impedance and moisture—to measure differences among the genotypes. The plants were grown under aerobic conditions in well-drained plastic bags filled with uniform sandy loam soil that were placed into polyvinyl tubes. The size of the tubes (1.0 m long by 0.2 m in diameter) was chosen to allow a good expression of root depth. The plants were watered three times per week with 500 mL of Yoshida's half-strength solution. The roots were assessed 45 days after sowing by measuring both the maximum extension of the root down the soil profile and, because of the sensitivity of such a measurement to the unusual behavior of any one root, the root dry weight at depth. The maximum extension of the nodal roots was determined by searching the extreme point reached by the roots beginning from the bottom of the soil column. Root weight by depth was measured by cutting the root column below a given point (generally below 30 cm), carefully sorting roots from soil under water, and oven-drying the roots. Root weight was preferred as a measure rather than total root length because of the ease of measurement (root length required a scanner and an image analyzer). A previous experiment showed that the two parameters were highly correlated ($r > 0.95$). An advantage of the "tube" technique is that it allows collection of the whole root system at once rather than just samples of it.

2.2 Genetic variability and selection of parents

A study was conducted with the Asian partners of the Upland Rice Research Consortium to assess the root depth of the upland varieties most commonly used as parents in their breeding programs (Courtois et al 1996). The set of varieties included some traditional varieties and a broad range of improved varieties or elite lines from various Asian countries. The overall results, with the entries organized by isozyme grouping, are presented in Table 1. The varieties used as drought-tolerant and drought-susceptible checks in the various countries differed in their rooting pattern (drought-tolerant lines with deeper roots), which confirmed the interest of the trait for the TPE.

For both maximum root depth and deep root weight, genotypic variance was highly significant. Variability between groups was observed. The variability within group was limited for group 2 (traditional aus varieties) and for the temperate component of group 6 (temperate japonicas). This showed that any variety of these groups could probably be chosen with some degree of confidence according to the value of its root system. Variability occurred within the japonica subspecies. But, for most traditional upland genotypes that are already deep-rooted, it may be difficult to find donors in the *Oryza sativa* species to increase rooting depth. The most reasonable breeding strategy might be to try to preserve the root traits in upland genotypes while improving their yield potential. A parallel study involving traditional varieties representing the six isozyme groups reached a similar conclusion (Lafitte et al 2001) and showed the extent of the variability in the whole species.

Table 1. Root depth of Asian upland rice varieties per varietal groups.

Varietal group[a]	Varieties sampled (no.)	Maximum root length (cm)	Within-group variability	Deep root dry weight (g)	Within-group variability
0	3	89.3 cd	*	0.173 c	*
1	26	87.3 d	**	0.178 c	**
2	8	106.1 a	ns	0.307 a	ns
Int. 1/2	6	93.2 cd	**	0.262 ab	*
6 trop.	50	101.1 ab	**	0.232 bc	**
6 temp.	5	95.2 bc	**	0.173 c	ns
Mean		96.5		0.220	
Cv (%)		10.8		43.4	

[a] 1 = indica, 2 = aus, 6 = japonica, trop. = tropical, temp. = temperate, 0 = intermediate, ns = nonsignificant; * and *= significant at 5% and 1% level, respectively. Means in columns followed by the same letter are not significantly different at the 5% level.

Source: Courtois et al (1996).

Sec. 4.4

2.3 Understanding the genetics of root depth

The use of molecular markers provides the opportunity to locate, with precision, the various genes controlling a trait, to evaluate their individual effects, and to manipulate them by selecting specific alleles at markers tightly linked with the genes (see Section 4.4). We analyzed the genetic control of root morphology in a doubled-haploid (DH) population of 105 lines derived from a cross between IR64, an indica shallow-rooted cultivar, and Azucena, a deep-rooted japonica variety (Yadav et al 1997). The experiment was conducted under greenhouse conditions. The broad-sense heritabilities of root depth and deep root weight were 0.77 and 0.60, reasonable values for use in a breeding program. The genetic analysis showed that both traits were quantitatively controlled. All interesting alleles came from the japonica parent. Quantitative trait loci (QTLs) were identified on chromosomes 1, 2, 3, 5, 6, 7, 8, and 9 for root depth and on chromosomes 1, 6, 7, and 9 for deep root weight. The QTLs on chromosomes 1, 6, 7, and 9 were common between both traits. Individual QTLs accounted for from 4% to 21% of the phenotypic variability, while the multiple QTL model accounted for 23% to 49%. The QTL on chromosome 7 was the only strong one, explaining from 15% to 21% of the phenotypic variability. Although additive × additive epistasis was not very frequent (close to 5% of the tested pairs), some nonallelic interactions between markers located on different chromosomes had magnitudes large enough to mask QTL detection.

Another team had already conducted the same type of work with a different population but a relatively similar protocol (Champoux et al 1995). Their study allowed the identification of QTLs expressed in both populations, therefore limiting the risk that they could be false positives. Among those, segments on chromosomes 1, 2, 7, and 9 appeared particularly interesting to us. Since then, new studies have confirmed these QTLs in additional populations (Kamoshita et al 2002).

2.4 Marker-aided selection for root depth

We decided to transfer the most interesting QTLs into the IR64 background to

- assess the effect of accumulating individual QTLs in a unique background,
- produce elite material with improved root characteristics and test it under stress, and
- test the possible effect of root depth under field conditions and the usefulness of such material.

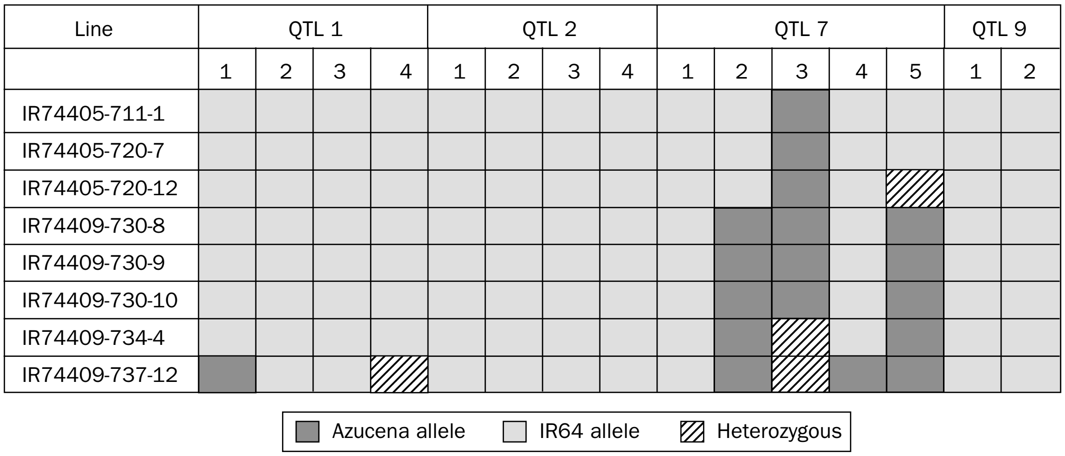
We chose four segments, on chromosomes 1, 2, 7, and 9, for introgression using marker-aided backcrosses. We used IR64 as the recurrent parent in three cycles of marker-assisted backcrosses.

The starting point for the backcross program was selected DH lines carrying the Azucena alleles at markers flanking one or several QTLs and a greater than average proportion of IR64 alleles in the rest of the background. The detailed procedure is given in Shen et al (1999). Two to five markers flanking the putative QTL were chosen for each segment. We selected the backcross progenies strictly on the basis of their genotypes at the marker loci in the target regions up to the BC_3F_2. We assessed the proportion of alleles remaining from Azucena in the nontarget areas of the BC_3F_2 plants, which was in the range expected for the backcross stage reached. Twenty-nine BC_3F_3 lines selected to represent various patterns of segregation within each segment were evaluated (Fig. 1). The techniques used to assess the root system of the lines were similar to those described earlier.

The results are presented in Table 2 (Shen et al 2001). Of the three tested near-isogenic lines (NILs) carrying target 1, one had significantly improved root traits over IR64. Three of the seven NILs carrying target 7 alone as well as three of the eight NILs carrying both targets 1 and 7 showed significantly improved deep root weight. Four of the six NILs carrying target 9 had significantly improved maximum root length. Five NILs carrying target 2 were phenotyped, but none had a root phenotype significantly different from that of IR64. A reanalysis of the raw data with the composite interval mapping technique, which has better resolution than the regression technique initially used, revealed two linked QTLs with opposite effects in this area. None of the tested lines had the favorable allelic combination at the two QTLs.

The phenotypic evaluation of BC_3F_3 families showed that the introgressed QTLs were expressed in the recipient background. The results confirmed that it was possible to treat each QTL as a Mendelian entity to produce lines better than the recurrent parent for both traits,

Fig. 1. Examples of molecular pattern for some near-isogenic lines carrying target region 7. 1, 2, 3, 4, 5 = markers used.



Line	QTL 1				QTL 2				QTL 7					QTL 9	
	1	2	3	4	1	2	3	4	1	2	3	4	5	1	2
IR74405-711-1	□	□	□	□	□	□	□	□	□	□	■	□	□	□	□
IR74405-720-7	□	□	□	□	□	□	□	□	□	□	■	□	□	□	□
IR74405-720-12	□	□	□	□	□	□	□	□	□	□	■	□	▨	□	□
IR74409-730-8	□	□	□	□	□	□	□	□	□	■	■	□	■	□	□
IR74409-730-9	□	□	□	□	□	□	□	□	□	■	■	□	■	□	□
IR74409-730-10	□	□	□	□	□	□	□	□	□	■	■	□	■	□	□
IR74409-734-4	□	□	□	□	□	□	□	□	□	■	▨	□	■	□	□
IR74409-737-12	■	□	□	▨	□	□	□	□	□	■	▨	■	■	□	□

■ Azucena allele □ IR64 allele ▨ Heterozygous

Marker-assisted selection resulted in some improvement in rice root characteristics.

Table 2. Results of the introgression of four QTLs for root traits.

Target	Number of different recombinants tested[a]	Number of lines with a phenotype significantly superior to that of IR64	
		Maximum root length	Deep root dry weight
1	3	0	1
2	5	0	0
7	7	1	3
1 + 7	8	0	3
9	6	4	0

[a] For each target, the tested lines could correspond to different recombinant patterns at the tested markers.

and that these traits were at least partly independently inherited. These results also pinpointed the difficulties of such work. The first issue was the quality of the QTL analysis: the most sophisticated method should be used to detect linked QTLs. Epistasis caused by interaction between genes should be evaluated and taken into account in the introgression strategy. As shown by a recent study (Ahmadi et al 2001), the transfer of interacting segments can be performed in the same way as for segments with additive effects, and this leads to the expected result. Preferably, major QTLs with a relatively limited confidence interval in their position should be used. Because the confidence interval is seldom going to be shorter than 20 cm anyway, the risk of linkage drag is high. In our case, some NILs were taller than IR64 and all had a decreased tiller number because of a likely cointrogression of linked QTLs. Lastly, performing a phenotypic evaluation at each step of the marker-aided backcross program would decrease the risk of keeping plants for which the QTL-marker linkage has been broken because of double recombination. Unfortunately, root depth evaluation is destructive.

3. Evaluation of the selections for root traits under field conditions

To assess whether the root depth observed under greenhouse conditions affected yield under stress, we tested the lines under field conditions with different water availability. A simple comparison among yields of the seven lines with increased root mass below 30 cm and 14 lines without increased rooting at depth showed greater yields in the deep-rooted set, both under well-irrigated upland conditions and in upland experiments with a 15–18-d period of water deficit at flowering (Fig. 2, Lafitte, unpublished results). The lines with greater yield were also significantly taller than IR64. Single-marker analysis of data for 58 introgression lines revealed negative effects on yield of Azucena alleles at markers RZ730 and RZ801 (chr. 1) and SDO419, RZ978, and RM248 (chr. 7). Azucena alleles at markers on chromosome 2 had positive effects on yield, but Azucena alleles at chromosome 9 markers had a negative influence on yield in aerobic conditions and positive effects on yield in a lowland experiment. Lines with Azucena alleles at target 1 markers had lower leaf relative water content in aerobic soil conditions. In contrast, Azucena alleles at markers on chromosome 9 had improved leaf relative water content. These results suggest that linkage drag was significant in this study. Several introgression lines were identified with yield significantly superior to that of IR64 (Fig. 2). These are candidates for further study to identify more accurately genetic regions that confer better performance and the mechanistic basis for greater yields in these lines.

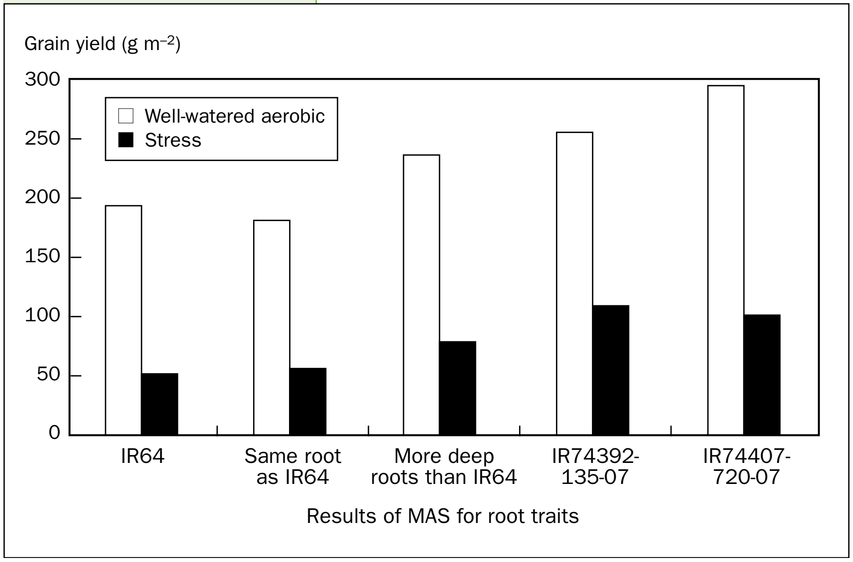


Fig. 2. Yields of lines selected using markers for root traits. The set of 14 lines that did not differ from IR64 for root mass in a greenhouse evaluation had grain yields similar to those of IR64 in both well-watered and droughted aerobic field plots. In contrast, the set of seven lines that had greater root mass below 30 cm in the greenhouse experiments had significantly greater yields than IR64. Two individual lines (not evaluated for root mass) had significantly greater yields than IR64.

4. What did we learn and what are the next steps?

Sec. 4.4

These results show that selection for complex traits is possible with a molecular approach. In standard breeding programs, however, QTL analysis in the populations of interest is seldom available. The advanced backcross QTL analysis method that allows the simultaneous discovery and transfer of interesting QTLs from an unadapted donor to an elite variety could then be a better approach (see Box 1, Section 4.4). We used the advanced backcross method to transfer QTLs for osmotic adjustment, a putative component of drought tolerance, from IR62266, an indica donor, into IR60080-46A, a japonica elite line with a deep root system. We have also used this approach to transfer root thickness and blast resistance from a traditional African variety into an elite Indian variety, Vandana. Although the phenotypic evaluation step can be quite delicate with such populations, the derived NILs may be extremely valuable material for farmers' direct use or for testing physiological hypotheses.

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Notes

Authors' addresses: B. Courtois, CIRAD-Biotrop, TA 40/03, Avenue Agropolis, 34398 Montpellier Cedex 5, France; R. Lafitte, International Rice Research Institute, DAPO Box 7777, Metro Manila, Philippines.

Color photos

1. Cultivar differences in leaf drying can be easily scored in the field if the drought period is long enough. These plots were without water for 26 d in a hot, dry climate (average temperature 28 °C, average PET 5.9 mm day^{-1}). Photo: R. Lafitte.

2. Contrasting strategies of drought response are found in IR61907, a rainfed lowland breeding line with a high level of osmotic adjustment (left), and Moroberekan, an upland cultivar with deep rooting (right). Leaf area in IR61907 was lower at onset of stress, and expansion quickly stopped. Moroberekan developed a large leaf area and, as stress became more severe, experienced severe leaf drying. Neither of these lines yielded well under this prolonged stress treatment. Photo: R. Lafitte.

3. Groundwater tubes and homemade tensiometers are adequate to monitor soil water conditions. Photo: R. Lafitte.

4

4. Scores of leaf rolling can be affected by maturity differences. When a line flowers under mild stress, it often shows less rolling than lines that have not yet flowered. These plants were irrigated 9 d before the photo was taken. Photo: R. Lafitte.

5

5. Plants that flower under stress exhibit desiccation of apical spikelets, discoloration of more protected spikelets, and poor exsertion of basal spikelets. All of these factors lead to reduced spikelet fertility in stressed panicles. Photo: R. Lafitte.

6. Sprinkler irrigation. Photo: B. Courtois.

8. Basin irrigation in leveled fields can provide uniform water application at a reduced frequency to generate continuous stress. Photo: R. Lafitte.

7. Drip irrigation can be used to apply different water treatments to adjacent plots. Photo: R. Lafitte.

9

9. Mid-season stress can be applied to lowland fields that have been established by direct seeding or transplanting. Photo: R. Lafitte.

10

10. In heavy clay soils, cracking begins about 3 weeks after the lowland paddy is drained. Photo: R. Lafitte.

11

11. By 5 weeks after draining, cracking is severe and symptoms of leaf drying are well advanced. Photo: R. Lafitte.

12. Timing and intensity of stress determine the impact of drought on the proportion of fertile spikelets. Increasing degrees of damage relative to the control (a) can be seen on panicles flowering after 8 d without irrigation (b), 12 d without water (c), and 18 d without water (d) for the cultivar IR64. Photos: R. Lafitte.

13. While all cultivars are affected by drought at flowering, the upland cultivar Apo shows greater tolerance. Control (a), 8 d without irrigation (b), 12 d without water (c), and 18 d without water (d). Photos: R. Lafitte.

14. One effect of drought is to delay flowering in most or all tillers. The plots on the left had water excluded as soon as the first flag-leaf ligules were visible (8 to 10 d before 50% anthesis in the control plots). The stressed plots show sparse and late flowering compared with the control plots (right), which are well advanced in grain filling. Photo: R. Lafitte.

15. Differences in leaf drying are apparent in these drip-irrigated 4-row plots. The plot on the left has been without water for 17 d, the next plot for 3 d, the next for 14 d, and the plot on the right is the control, irrigated earlier the same day. Photo: R. Lafitte.

16. Desiccated spikelets occur when hot, dry conditions coincide with flowering. Photo: R. Lafitte.

17. Upland rice with sprinkler irrigation in Brazil. Photo: B. Courtois.

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