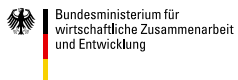




# Enhancing the use of crop genetic diversity to manage abiotic stress in agricultural production systems

*23–27 May 2005, Budapest, Hungary*

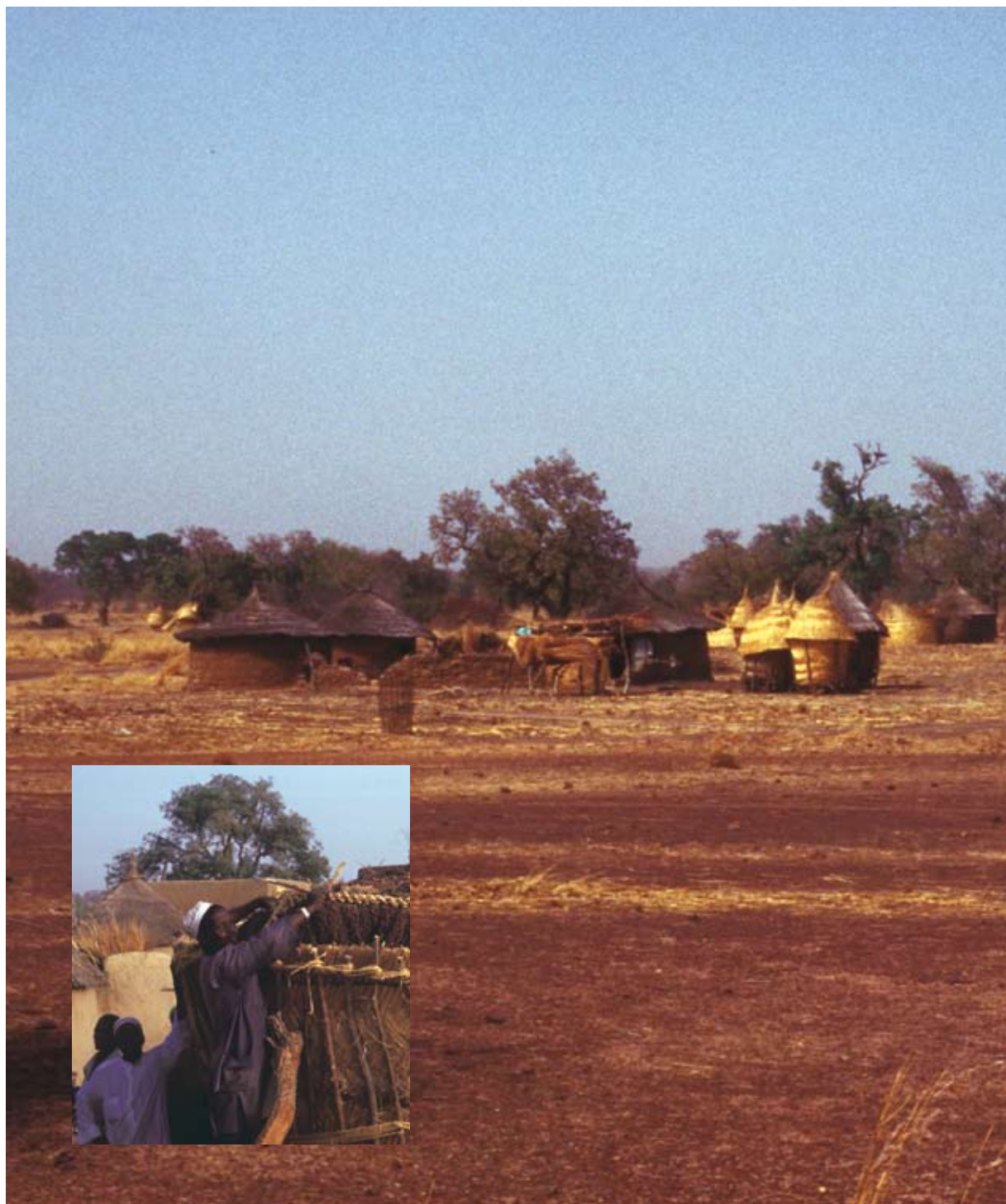
**D. Jarvis, I. Mar and L. Sears editors**



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## I. Introduction

*Devra Jarvis and Toby Hodgkin*

Diversity can provide one means of sustaining and improving the livelihoods of farmers where or when environmental conditions are unfavourable in production systems. Much of the world's agricultural biodiversity is found in environments marginal for agricultural production. It is in such environments where management of high levels of diversity can become a central part of the livelihood management strategies of farmers and the survival of their communities. Loss of such diversity or genetic choices diminishes farmers' capacities to cope with extreme temperatures, drought and salinity. Farmers in stress-prone areas have to take particular care not to introduce non-adapted material which often fails and can lead to erosion of the desired characteristics in local varieties through unwanted crossing. Thus, in the face of constant vulnerability to loss of key stress-resistant types, the management of appropriate diversity of a specific crop constitutes a significant component of livelihood strategies of farmers in highly stressed production environments.

Within the global project, 'Strengthening the Scientific Basis of *In Situ* Conservation of Agricultural Biodiversity On-farm', and other projects concerned with the conservation and use of local crop diversity on-farm, national and international partners have developed tools to measure the amount and distribution of crop genetic diversity on-farm, to analyze the processes that maintain this diversity and to calculate values of this diversity to farmers and other stakeholders. Yet, there are still important unknowns concerning the diversity in local varieties managed by farmers subject to significant abiotic stress. These include:

- Whether within farmers' use of diversity for managing abiotic stress: Does the use of stress-tolerant germplasm 'cost' lower yield? In the stressed environment? In benign environments? In both?
- How different are the local crop varieties—phenotypically and genetically—with respect to resistance to the specific traits conferring stress tolerance or resistance?
- Do the tolerant or resistant populations have sufficient diversity with respect to other characteristics, thus helping farmers to exploit their potential to develop material with further desirable characteristics such as pest resistance, nutritional aspects, grain quality?
- Do farmers manage such materials in a specific way to prevent dilution of their stress-resistance characteristics, and does this limit adaptability?

From 23 to 27 May 2005, a workshop was held in Budapest, Hungary to refine these questions and to define areas where more basic research that uses tools which assess diversity, its structure and changes would be needed. During the workshop, the participants from Burkina Faso, Morocco, Zimbabwe, Mali, Peru, Vietnam, Tunisia, Hungary, Bolivia, Nepal, Australia, Italy and the US presented their ideas and information from their own work on enhancing the use of crop genetic diversity to manage abiotic stress. The following papers in this proceedings document the partners' presentations and the output of the discussion groups.

## II. Abiotic Stress

### Genetic features of populations from stress-prone environments

*Anthony Brown and Loren Rieseberg*

#### **Introduction**

We live in a momentous period for deploying plant genetic resources to meet abiotic environmental challenges to agricultural productivity and human well-being. Several factors are converging to bring this about. First, the broad problem of crop loss due to abiotic stress is global in scope, truly impacting worldwide. Typical of statements in the current literature is the following quotation:

“Abiotic stress is the primary cause of crop loss worldwide, reducing average yields for most major crop plants by more than 50%... Drought and salinity are becoming particularly widespread in many regions, and may cause serious salinization of more than 50% of all arable lands by the year 2050.” (Wang et al. 2003)

Environmental stresses such as erratic and insufficient rainfall, extreme temperatures, salinity, alkalinity, aluminium toxicity, acidity, stoniness and others limit yield and productivity of many cultivated crop plants. Not only are such problems serious today, it seems they are inevitably worsening. For example, large tracts of productive land, and indeed whole river basins, in Australia are suffering secondary salinization due to rising subsurface water levels that have followed from vegetation clearing and excessive irrigation (Munns 2005). These practices we now know to be unsustainable in the longer term.

A second ingredient in the present situation is the store of knowledge in established fields of plant biology and agroecology. There is the experience of plant collectors and breeders who have found and used sources of tolerance to abiotic stresses among and within crop species. Also plant physiologists have determined cellular, tissue and whole plant responses, or morphological or phenological shifts that help plants ameliorate or avoid the impact of detrimental environmental factors. Plant ecologists have described ecotypic variation as adaptive responses to intense selection gradients, particularly in soils.

Teshome et al. (2001) reviewed the published research on variation in farmers' traditional varieties or landraces of cereals and pulses in their centres of origin. The review was concerned with studies of the influence of geographic, biotic, abiotic and human factors that maintain genetic diversity and population differentiation in traditional cultivars still grown. Some 42% of studies focused on geographic separation at various scales (between countries, regions or farms). Disease and pests were the diversifying factors in about 10% of studies. Abiotic gradients or mosaics in altitude, climate, soil or field size were studied in 29% of cases, and abiotic extremes (aridity, cold, salinity, waterlogging) in 14%, leaving only 6% on farmers' selection criteria. Many descriptive reports measured variation for either genetic markers or morphology. However, fewer reports sought to analyze the function of the diversity and the key factors that maintain it. Furthermore, most studies examined divergence between populations; fewer have focused on variation within individual populations. There is clearly scope to deepen and enrich the research agenda in this area.

The third source of excitement—and for some the most promising one—is the array of powerful tools of modern plant molecular biology in functional genomics and gene expression profiling in stress response (Cushman and Bohnert 2000). These include the use of microarrays and genome mapping to detect and precisely manipulate stress-response genes in breeding programmes, and in the growing ability to engineer new or 'foreign' genes into plant genomes. Great advances have been made in recent years in understanding the molecular basis of plant response and plant tolerance to drought and other stresses. Hundreds of drought-responsive genes have been identified and the function of some has



been resolved at the cellular level (e.g. Seki et al. 2005). Despite this, a huge gap remains between the findings at the molecular level and the application of this knowledge at the whole plant level for improved yield on-farm. As Flowers (2004) commented, despite 10 years of research using transgenic plants to alter salt tolerance (and the gain in basic knowledge this has produced), the value of this approach has yet to be established in the field.

It is well beyond our aim here to synthesize all these contributing elements. Rather we will focus on the research required to understand the management of varietal diversity on-farm, particularly where subsistence farmers must meet, maintain and indeed improve their livelihood in the face of abiotic stresses to crop production.

### ***The link between stressed environments and landrace diversity***

Although they are yield limiting, environmental stresses influence the generation and maintenance of intraspecific diversity for increased capacity to cope with them. Extreme environments are likely to select for a suite of characters that allow survival, a co-adapted complex. Hence it is particularly important to ask whether local landraces in extreme habitats possess specialized tolerant genotypes, rather than those that are broadly adapted and cope by phenotypic plasticity.

In rice, for example, abiotic stresses that are major limits on rice production in rain-fed environments (45% of global rice area) include water deficit, cold, salinity and mineral deficiencies. For drought, “farmers have been selecting those plants that survived drought events for centuries, and there is a wealth of genetic variation for response to water deficit among traditional cultivars. There are few examples, however, of improved cultivars that combine acceptable yield potential and drought tolerance” (Lafitte et al. 2004).

In many instances, breeders have successfully turned to traditional varieties grown in stress-prone environments for sources of resistance. One prevailing view on current geographic patterns of diversity in major crops is that the varieties developed by modern plant breeding have largely supplanted traditional varieties in the major central productive areas worldwide (Frankel et al. 1995). If traditional varieties still linger, it is generally at the margins, in poorer areas, where the reach of plant breeding has been less. It is argued that modern programmes have tended to emphasize wide adaptability rather than specialist breeding. Indeed it is the lack of breeders for rarer extreme environments which explains the limited options for farmers in such environments (Frankel et al. 1995).

On the other hand, farmers in stress-prone areas have to take particular care not to introduce non-adapted material which often fails in adverse conditions, and could lead to erosion of the desired characteristics in their local varieties through unwanted crossing. Thus, in the face of constant vulnerability to loss of key stress-resistant types, the management of appropriate diversity of a specific crop constitutes a significant component of livelihood strategies of farmers in highly stressed production environments.

Yet, as Fischer (pers. comm.) has pointed out, there is no reason why modern varieties of major cereal crops cannot pick up the useful abiotic stress traits from traditional varieties and produce even better varieties which farmers might prefer to grow over traditional varieties. Thus the management of crop diversity by farmers in stress-prone areas must address livelihood maintenance in extreme environments, *in situ* conservation of adapted diversity, and ideally varietal improvement by using local materials as sources of resistance.

### ***The genetic structure of populations from stress-prone environments***

How does the genetic structure of populations from stress-prone environments compare and contrast with that of related ones from benign environments? To address this overall question we make a series of subsidiary comparisons, and summarize them as questions in Box 1.

### **Box 1. The genetic nature of landrace populations from stressed environments.**

1. Are they more or less polymorphic than related ones from benign environments? The question applies to both neutral genetic markers and selected traits.
2. Is performance (yield or quality) poorer than stress-sensitive populations when grown in a favourable environment? Does cost vary with specificity of tolerance?
3. Are they of hybrid origin or more heterozygous than populations from benign environments? Are hybrids able to tolerate abiotic stress better than their parents?
4. Are marginal populations more divergent among themselves and from central ones, than are central ones themselves for neutral or marker alleles? Do they have smaller sizes and reduced geneflow?
5. Is there clear adaptive divergence in gene expression? Does gene expression profiling provide a meaningful way of assessing biotic diversity?
6. Is allelic variation at relatively few major genes the main source of adaptive response, or is response due to the accumulation of many QTLs?
7. Is the adaptive response one of stress-avoidance, or direct improved tolerance?

### **1. Are stressed populations more—or are they less—polymorphic than related ones from benign environments?**

Few if any data are collated for landrace populations to answer this question. One example is the comparison of landrace genetic structure in three contrasting regions in Nepal: the agriculturally highly productive lowland (Bara), the mid-hill (Kaski), and the high mountain region (Jumla), which is subject to cold stress (Gyawali et al. 2005). Table 1 summarizes landrace richness as reflected by the number of farmer-named distinct landraces that the average household is using. For the dominant crop rice, most farmers use only one landrace in contrast to the other two less-stressed regions, reflecting how dependent they are on cold-tolerant genotypes. Estimates of molecular marker (microsatellite) variation within the landraces reflect the same pattern of diversity. Populations at the margin are noticeably poorer in diversity. This pattern applies also for cucumber, a small-scale vegetable satisfying a variety of uses. Interestingly, however, the pattern for finger millet differs, with households in the cold-stressed region growing more landraces which presumably reflects the increased cold-tolerance, importance and use of this grain in the more marginal situations. Millet diversity was least in the benign, highly productive region where advanced varieties of rice prevail.

Studies of the genetic structure of natural wild populations have tended more often to frame it as a question of whether 'marginal' populations are less variable than central ones. A typical example is the species *Corylus avellana* (Betulaceae) in which Persson et al. (2004) found that marginal populations were less polymorphic for allozyme allelic richness, evenness and genotype diversity. Greater population divergence among the marginal populations accompanied this trend. The authors invoked historical effects (post-glaciation colonization, bottlenecks of population size and genetic drift) interlinked with selection as the explanation. However the diversity patterns for selectively neutral genetic markers such as allozymes may be poor indicators of polymorphism at loci and traits that are under selection in marginal populations.

**Table 1. Richness and diversity of rice and finger millet landraces in three regions in Nepal (Data modified from Gyawali et al. 2005).**

| Community or region                                 | Bara<br>Low land | Kaski<br>Mid-hill | Jumla<br>High mountain |
|---|------------------|-------------------|------------------------|
| Rice ( <i>Oryza sativa</i> )                        |                  |                   |                        |
| Number of named landraces per household             | 3.82             | 4.51              | 1.09                   |
| Average per farm gene diversity for microsatellites | 0.4              | 0.39              | 0.17                   |
| Finger millet ( <i>Eleusine corocana</i> )          |                  |                   |                        |
| Number of named landraces per household             | 1.06             | 1.72              | 1.36                   |
| Cucumber ( <i>Cucumis sativus</i> )                 |                  |                   |                        |
| Number of named landraces per household             | 1.00             | 1.20              | 1.11                   |

As postulated in this example, geographic marginality is indicative of stressed situations at the ends of ecological gradients of temperature, and the broad-scale distribution of particular crop species. In such cases population sizes may be low, and migration reduced. Overall small sizes and low migration may lead to reduced local polymorphism. Alternatively, adaptive specialization and selection for a limited array of genotypes might account for reduced polymorphism. In practice it may be impossible to disentangle both these causes. Geographic marginality need not necessarily equate to ecological marginality. Climatic and edaphic extremes for a species will often be located within its geographic range.

## 2. Is yield performance compromised by adaptation to stress-prone habitats?

This question relates to the possible existence of yield costs or trade-offs for stress-tolerant characters. Such costs may be greater for tolerances obtained from wild related species compared with those that are present in landraces already. They may also be greater for mechanisms that condition more 'general tolerance' as opposed to specific tolerances. The relative performance of both tolerant and sensitive populations in both stressed and benign environments requires assessment.

## 3. Are populations adapted to stress more heterozygous than their benign counterparts, and if so, is this because hybrids are better able to tolerate abiotic stress?

Populations of recent hybrid origin may adapt to stress through heterosis, i.e. the pronounced extra vigour from wide crosses, or from segregation in the F<sub>2</sub> and subsequent generations of a wide spectrum of variation. The potential contribution of heterosis to abiotic stress tolerance is exemplified by invasive allopolyploid (Ainouche et al. 2003) and clonal diploid hybrids (Gaskin and Schaal 2002; Moody and Les 2003). In the absence of processes that fix heterozygous genotypes—such as allopolyploidy, apomixis and clonal spread—heterosis will be transitory. However, selection can stabilize recombinant genotypes that are able to occupy extreme and therefore relatively vacant habitats and less competitive situations. For example, hybrid sunflower species of the genus *Helianthus* have been able to colonize extreme sand dune, desert floor and salt marsh habitats by combining adaptive combinations of parental alleles (Rieseberg et al. 2003).

Sugarcane is an example of one crop in which interspecific hybridization (in this case the incorporation of genes from wild relatives into the 'noble' cane (*Saccharum officinarum*)) resulted in hybrid vigour, yield increase, stress tolerance, resistance to some diseases and dramatic range expansion with notable increased adaptation to marginal environments (Simmonds 1976; Shenck et al. 2004).

Yet, in general, interspecific hybrids in wild populations often show a contrast between increased abiotic resistance, but with increased predation (or infection) than their parental species, as they inherit susceptibility from two sources. In the studies that Fritz et al. (1999)

reviewed, the overall trend in the 56 field studies of interspecific hybrids of plants was towards increased herbivory on the hybrids, and in the clear majority of the 28 studies on fungal disease, the hybrids retained the same as, or increased susceptibility over the more susceptible parent. They concluded that hybridization can often disrupt the normal resistance of plant species to their parasites. It may be necessary to test for greater susceptibility to biotic pressures with elevated hybridity in the adverse environments themselves, where stress tolerance may be shown when those environments happen to act to suppress the biotic pressures in question.

#### **4. Are marginal populations more divergent for neutral alleles, i.e. is there reduced geneflow?**

Data from marginal salt-adapted sunflowers indicate that while differentiation is higher, and effective migration rates are lower for genes under selection, migration rates at other loci do not differ between marginal and central populations (Kane and Rieseberg, unpubl.<sup>1</sup>). However, similar studies of other taxa will be required to assess the generality of this pattern.

#### **5. Is there clear adaptive divergence in gene expression in stressed populations compared with controls?**

Such a divergence would offer the possibility that gene expression profiling may provide a meaningful way of assessing biotic diversity. This would offer a future rapid assessment of germplasm for tolerance. Yet there are major technical and ground-truthing challenges to solve, particularly to validate profiling as a predictive tool.

How do we most effectively reveal genes that contribute to abiotic stress tolerance or avoidance? Breeding programmes have generally relied on results from field evaluations for identifying populations or genotypes that are most likely to contain alleles of interest. However, we also know that maladapted material may contain cryptic alleles for tolerances (or other traits) that can only be revealed by crosses with divergent genotypes (Tanksley and McCouch 1997). While it does seem worthwhile to identify lines with valuable cryptic variation, crossing studies are time-consuming, and it might be possible to avoid this step through expression profiling to detect differences in gene expression among lines with seemingly similar phenotypic stress responses.

#### **6. To what degree are major genes the source of the adaptive response, or how much of tolerance is achieved by the accumulation of many minor QTLs?**

#### **7. Is the adaptive response one of stress-avoidance or direct improved tolerance?**

Perhaps the answer to this question does not matter. For breeder and farmer alike the arbiter of tolerance is yield, whichever way it is achieved. However, the question of tolerance versus avoidance may well be important in situations where there are trends towards increasing severity of stress with time.

### ***Comparison and contrast between abiotic and biotic stress and plant response***

In considering the research agenda for investigating the use of genetic diversity to enable farmers to cope with abiotic stress factors in their production systems, it is instructive to compare and contrast managing diversity for abiotic versus biotic stresses. We do so here under four headings: the nature of and variation in the two kinds of environmental stress, the plant and its response, patterns of change in time, and the issues as seen by farmers.

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<sup>1</sup> Kane, N.C., and L.H. Rieseberg. Selective sweeps reveal candidate genes for adaptation to drought and salt tolerance in common sunflower, *Helianthus annuus*.

### Environmental stress

- The scale of variation in the stress, both in time and in space is greater for abiotic stresses.
- The 'graininess' of environment (Levins 1968)—how the individual experiences both temporal and spatial variation in the environment—coarse-grained (abiotic) and fine-grained (biotic).
- The degree of abiotic stress is less affected by the plant condition. The health and vigour of the host plant affect the amount of disease damage. However the response to both kinds of stress is likely to vary with growth stage.
- Nevertheless, interplay between the abiotic and the biotic is frequent as both comprise the environment of the plant. Examples of such interplay are salt-sensitive symbionts in legumes, ameliorating drought stress response in cereals by breeding resistance to root diseases.

### Plant and its response

- The plant is the object in the case of abiotic stresses with little impact on the degree of stress, whereas it is the host for diseases and a food source for pests.
- Stresses differ for the relative importance of major genes as the source of the adaptive response, versus the accumulation of several to many minor genes. Heavy metal and aluminium tolerances, as well as qualitative 'gene for gene' responses to biotic stress tend to be major genes. In contrast, drought tolerance and quantitative response to biotic stresses have a more complex genetic basis.
- The relative importance of divergence versus local polymorphism differs, with the former more prominent for abiotic stresses. This in theory relates to differences in graininess, temporal variation in the environment or frequency-dependent selection.
- Phenotypic plasticity, avoidance, and multiple mechanisms of tolerance and avoidance that may act at different stages of the life cycle means that resistance to abiotic stress may combine or pyramid more effectively than will tolerances for biotic stresses.
- Experimental assays of resistance or tolerance vary in their complexities. For abiotic stress, difficulties arise in the repeatability of measures, the multiplicity of mechanisms, and the meaningfulness extrapolations from the test environment to the field (Munns 2005). In biotic stress, assay responses are often race-specific. Therefore the relation between virulence structure of the assay compared with that of the native pathogen populations is key.

### Temporal change

- A co-evolutionary feedback does not operate for abiotic stresses. Changes in the crop genetic make-up to cope with the stress will not directly bring about a change in stress levels, in contrast to the pathogen populations.
- Secular trends with time are at a slower pace for abiotic stresses. Temporal gradients of virulence or prevalence in pathogens can be very rapid compared with climate change and unsustainable land-use patterns.

### Farmer perspective

- The ease with which farmers recognize the major yield constraints is likely to differ widely between kinds of stress. Differences in clarity and specificity of plant symptoms, climatic changes between years, prominence of associated organisms, farmer knowledge and experience differ among stresses.
- The scope and role of diversity strategies such as mixtures and interbreeding differs between abiotic and biotic stress. For abiotic stresses there may be less scope for diversity within populations and greater emphasis on using tolerant varieties.
- Intellectual property due to the potential economic value of resistance sources may vary depending on the effectiveness of the resistance it confers, the value of the crop and the

geographic extent of the stressor. The potential economic value of recognized and genetically defined sources of resistance or tolerance to abiotic stresses raises the issue of benefit-sharing with the originating farmers, particularly if they come from poor marginal areas.

Bellon et al. (2003) surveyed farmers from Oaxaca, Mexico as to the positive or negative selection criteria they use to choose among a large number of samples of landraces. The criteria were collated to form a check list of farmer selection criteria. The whole collection was grown in a diversity trial and groups of farmers were asked to rate the entries for the possible criteria. The data indicate the relative importance of the criteria as reasons for varietal choice and are summarized in Table 2. Noteworthy in the overall trends was the very low prominence in farmers' positive responses for resistance to abiotic and biotic pressures. Rather, the attractiveness for specific culinary uses dominated. Yield attributes were also more important, although it could be argued that yield was a surrogate for resistance to local abiotic stresses that the standing crop had experienced. Biotic pressures that farmers witnessed as grain loss during storage were also important. The authors stressed the general appreciation of diversity by farmers and preparedness to use diversity for their welfare. These are the first steps in developing an understanding of the selection regimes used by farmers on-farm.

**Table 2. Farmers' perceptions of the positive and negative characteristics associated with landraces collected in Oaxaca, Mexico (data from 213 maize farmers, Bellon et al. 2003, summarized by kinds of traits).**

| Farmers' perception           | Percentage cited as: |          |
|-------------------------------|----------------------|----------|
|                               | Favourable           | Negative |
| Consumption                   | 44.2                 |          |
| Yield                         | 34.8                 | 37.0     |
| Early maturity                | 10.5                 |          |
| Saleable                      | 2.2                  |          |
| Ease of shelling              | 1.7                  |          |
| Abiotic stress, adaptedness   | 3.3                  | 14.8     |
| Biotic stress – during growth | 1.1                  |          |
| Storage pests of grain        | 2.2                  | 44.4     |

### Conclusions

To counter biotic stress, the overall goal for research on farmers' use of diversity is the reduction in genetic vulnerability. The impetus towards the goal lies not so much in the current situation on-farm, but in what might lie just ahead for the farmer, because pests and pathogens are bound to evolve in response to present productivity. Since we expect pathogen evolution to happen and to be diverse, diversity in the host will be needed to counter it.

With abiotic stress, the emphasis shifts to the here-and-now. The focus is on those margins where such stresses currently limit crop production and threaten farmer livelihoods. We want genes for today, rather than genes for tomorrow. Yes, but is there a need for diversity *per se*? Is it adequate to search for the responsive genes in wild plants that confer adaptation in extreme halophytes or xerophytes, detect and extract them by molecular approaches? One answer stems from what has been learnt from surveys of stress response among accessions in germplasm collections and the physiological analysis of the tolerances detected. Tolerance or resistance is commonly a complex of characters combining mechanisms of both direct tolerance and indirect avoidance. These different mechanisms can also differ in their associated yield costs. Thus a diversity of sources of stress tolerances is

needed as the raw material for combining or pyramiding to achieve improved tolerance, and for crossing with high-yielding but sensitive populations. Some basic research questions whose resolution would lead to progress toward this goal are listed in Box 2. This implies a major role for participatory approaches to plant improvement.

**Box 2. Questions to guide research on the role of genetic diversity in coping with abiotic stress.**

1. Local varieties harbour considerable genetic diversity. For those from stress-prone or marginal environments, is this diversity 'relict' or 'adaptive'? i.e. is it left over from the past and still present merely because high-yielding modern varieties have yet to be bred for those areas, or does it confer adaptedness to local environments?
2. How different are local crop varieties—phenotypically and genetically—with respect to the specific traits conferring stress tolerance or resistance? What is the genetic basis of the resistance (co-adapted complexes of many genes, or a small number of major genes)?
3. Within farmers' use of diversity for coping with abiotic stress, what are the 'costs' in terms of loss of yield (if any) for the use of stress-tolerant germplasm?
4. How diverse are tolerant or resistant populations with respect to other characteristics (pest resistance, nutritional needs, grain quality and yield) that enable farmers to exploit their potential?
5. What special management (if any) do farmers use specifically to prevent dilution of the stress resistance characteristics of local varieties?

Three important perspectives lend urgency to the task of using diversity to mitigate abiotic stress. These are:

- Abiotic stresses are rarely singular. More likely they impact on plant populations as a set of interacting multiple stresses present in a given environment. These interactions will include those with biotic stresses such as disease, pests and the lack of beneficial symbionts and pollinators. The need for genetic diversity to cope with each of these interacting stresses gives a new impetus on the concern about the loss of genetic resources and genetic vulnerability.
- Climate change and environmental deterioration have set up a series of secular changes in the intensity of abiotic stresses with time. The lesson from this realization is that future genetic options are at a premium if we are to meet such changes.
- Abiotic stress impacts most clearly in marginal environments and therefore is globally linked with poverty and exacerbates socioeconomic and political problems. Mitigating the effects of stress on productivity with diversity strategies is a key to poverty alleviation.

**References**

- Ainouche, M.L., A. Baumel, A. Salmon and G. Yannic. 2003. Hybridization, polyploidy and speciation in *Spartina* (Poaceae). *New Phytologist* 161:165–172.
- Bellon, M.R., J. Berthaud, M. Smale, J.A. Aguirre, S. Taba, F. Aragón, J. Díaz and H. Castro. 2003. Participatory landrace selection for on-farm conservation: An example from the Central Valleys of Oaxaca, Mexico. *Genetic Resources and Crop Evolution* 50:401–416.
- Cushman, J.C. and H.J. Bohnert. 2000. Genomic approaches to plant stress tolerance. *Current Opinion in Plant Biology* 3:117–124.
- Flowers, T.J. 2004. Improving crop salt tolerance. *Journal of Experimental Botany* 55:307–319.
- Frankel, O.H., A.H.D. Brown and J.J. Burdon. 1995. *The Conservation of Plant Diversity*. Cambridge University Press, UK.

- Fritz, R.S., C. Moulia and G. Newcombe. 1999. Resistance of hybrid plants and animals to herbivores, pathogens and parasites. *Annual Review of Ecology and Systematics* 30:565–591.
- Gaskin, J.F. and B.A. Schaal. 2002. Hybrid *Tamarix* widespread in US invasion and undetected in native Asian range. *Proceedings of the National Academy of Sciences USA* 99:11256–11259.
- Gyawali, S., B.K. Joshi, P.R. Biswokarma, A.H.D. Brown, D. Jarvis and B.R. Sthapit. 2005. The amount and distribution of local crop diversity in three ecophysiological regions of the *in situ* conservation project in Nepal. Pp. 2-14 *in* On-farm Conservation of Agricultural Biodiversity in Nepal. Volume 1 (B.R. Sthapit, M.P. Upadhyay, P.K. Shrestha and D.I. Jarvis, eds.). IPGRI, Rome.
- Lafitte, H.R., A. Ismail and J. Bennett. 2004. Abiotic stress tolerance in rice for Asia: progress and future. Pp. 1–17 *in* Proceedings of the 4th International Crop Science Congress, Sept 2004, Brisbane Australia. [www.crops-science.org.au](http://www.crops-science.org.au).
- Levins, R. 1968. *Evolution in Changing Environments*. Princeton University Press, Princeton NJ.
- Moody, M.L. and D.H. Les. 2002. Evidence of hybridity in invasive watermilfoil (*Myriophyllum*) populations. *Proceedings of the National Academy of Sciences USA* 99:14867–14871.
- Munns, R. 2005. Genes and salt tolerance: bringing them together. *New Phytologist* 167:645–663.
- Persson, H., B. Wilden, S. Andersson and L. Svensson. 2004. Allozyme diversity and genetic structure of marginal and central populations of *Corylus avellana* L. *Plant Systems and Evolution* 244:157–179.
- Rieseberg, L.H., O. Raymond, D.M. Rosenthal, Z. Lai, K. Livingstone, T. Nakazato, J.L. Durphy, A.E. Schwarzbach, L.A. Donovan and C. Lexer. 2003. Major ecological transitions in annual sunflowers facilitated by hybridization. *Science* 301:1211–1216.
- Schenck, S., M.W. Crepeau, K.K. Wu, P.H. Moore, Q. Yu and R. Ming. 2004. Genetic diversity and relationships in native Hawaiian *Saccharum officinarum* sugarcane. *Journal of Heredity* 95:327–331.
- Seki, M., J. Ishida, M. Nakajima et al. 2005. Genomic analysis of stress response. Pp. 248–265 *in* Plant Abiotic Stress (M.A. Jenks and P.M. Hasegawa, eds.). Blackwell Publishing, Oxford, UK.
- Simmonds, N.W. 1976. Sugarcane. Pp. 104–108 *in* Evolution of Crop Plants (N.W. Simmonds, ed.). Longmans, London and NY.
- Tanksley, S.D. and S.R. McCouch. 1997. Seed banks and molecular maps: unlocking genetic potential from the wild. *Science* 277:1063–1066.
- Teshome, A., A.H.D. Brown and T. Hodgkin. 2001. Diversity in landraces of cereal and legume crops. *Plant Breeding Reviews* 21:221–261.
- Wang, W., B. Vinocur and A. Altman. 2003. Plant response to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta* 218:1–14.



### III. Drought Stress

#### Diversity of Moroccan local faba bean landraces for reaction to drought stress

*Mohammed Sadiki*

##### **Introduction**

In Morocco, as elsewhere across the Mediterranean region, drought is the most important environmental multidimensional stress and a permanent constraint affecting the security and sustainability in agricultural production which is highly dependent on the amount of rainfall and its distribution during the crop cycle (Ceccarelli and Grando 1996). Over 50% of the precipitation is concentrated on only 15% of the country's area; average annual rainfall is 340 mm varying from more than 500 mm in the northern part to less than 150 mm towards the southeast.

Faba bean, the major grain legume in Morocco, is one of the crops very susceptible to drought (Day and Legg 1983). As a winter-sown crop it can experience both intermittent drought stress caused by breaks in winter rainfall and terminal drought stress resulting from receding soil moisture. Crop production is drastically affected by both the amount and pattern of rainfall distribution (Grashoff 1990). Irrigation induces a high yield increase of this crop (Hebblethwaite et al. 1977; French and Legg 1979; Farah 1981).

Cultivating tolerant genotypes is the most attractive approach to attenuate the negative effects of drought on crop production. Drought escape, dehydration avoidance, and drought tolerance are the plant strategies to cope with the physiological events associated with drought (Blum 1988). These mechanisms can involve different cell pathways resulting in the expression of stress-response genes. Drought tolerance of a crop is seen as the minimum difference in yield between stress and non-stress environments (Fischer and Maurer 1978; Langer et al. 1979; Rosielle and Hamblin 1981; Blum 1983, 1988). It is also defined as the relative yield of a genotype compared with other genotypes subjected to the same drought stress (Hall et al. 1993).

Much of the Moroccan agricultural biodiversity is found in rain-fed areas and in marginal environments subject to recurrent drought (Sadiki and Jarvis 2005). In these environments management of high levels of diversity may be one of the few resources available to farmers to cope with extreme temperatures and drought. The Moroccan local faba bean populations express a great genetic diversity for many morphological, agronomic and physiological traits (Sadiki 1990; Mehdi 1994; Berrada 1995; Alami 1995; Lazrak 1995). However, no study has been carried out on this material for testing its reaction to drought stress. Moreover, studies on the performance of faba beans under drought are limited, and those available were carried out with few genotypes (Dantuma and Grashoff 1984; Saxena et al. 1986; Grashoff 1990). Genotypic differences in drought tolerance levels have been reported in this crop. Stelling et al. (1994) observed quantitative differences in collections of faba bean inbred lines screened for drought tolerance.

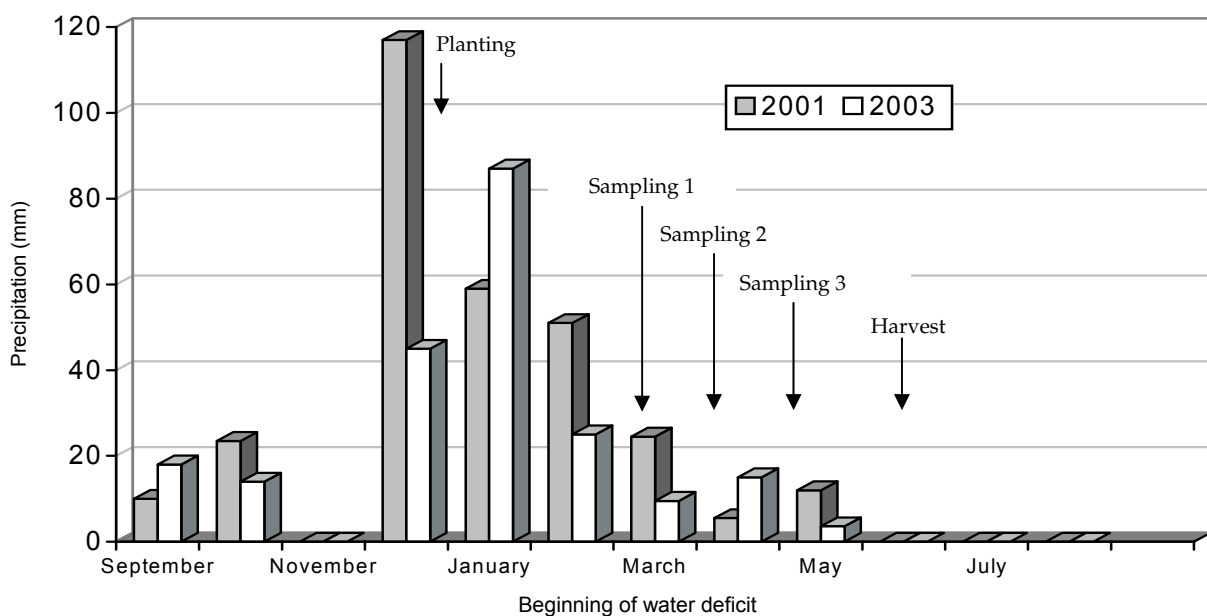
Because of insufficient genetic variability for traits like drought tolerance, extensive evaluation of local genetic diversity of faba bean, an ancient crop in Morocco, is an approach for identifying adapted genotypes, thereby responding to farmers' need and providing useful germplasm sources for breeders.

The objective of this study was to assess the genetic diversity within a collection of local populations of faba bean for tolerance of water stress and to develop indices for identifying tolerant genotypes.

### Material and methods

The field experiments were conducted in 2001 and 2003 seasons at the Experimental Farm, Ain Dick, SOGETA which is located 20 km south of Rabat. The plant material comprised 90 diverse local faba bean populations belonging to 10 local varieties (9 populations per variety). These populations were collected from farmers' fields throughout the crop-production areas in Morocco between 1995 and 2000. The 9 populations of each variety were sampled from different farmers.

The experiment was laid out as a split-plot design with 4 replications under two water treatments: moisture non-stress (MNS) and moisture-stress (MS) treatments. In the MNS treatment, the soil moisture was maintained, through a drop irrigation system, at the field capacity to avoid any water stress. The MS treatment relies on rain and limited irrigation was supplied to avoid chronic stress inducing plant loss. The precipitation profiles recorded during the 2 years of experiments are presented in Figure 1.



**Figure 1. Volume of precipitation recorded at the Ain Dick Experimental Farm, SOGETA, during the trial time, 2001 and 2003.**

Each accession was grown in a 6 × 3 m plot with 70-cm inter-row and 14-cm interplant spacing. From each plot 5 plants were sampled 2, 3, and 4 months after plant emergence (Figure 1). Immediately after harvest, plants were weighed and oven-dried at 70°C for 24 h to determine dry weight. Water content was determined as  $WC = [(fresh\ weight - dry\ weight) \times 100] / fresh\ weight$ . At maturity, 10 plants were randomly sampled from each plot and used to measure plant growth traits and yield-associated parameters.

Different statistical analyses were performed on the collected data. Drought tolerance was assessed using different parameters. The parameters, based mainly on the data of grain yield/plant, are as follows:

- Yd/Yi Ratio of yield in dry treatment (Yd) to yield in irrigated treatment (Yi) expressed in%.
- AR Absolute reduction in yield due to stress, computed as the difference between Yi and Yd (Yi – Yd).

- RR Relative reduction in yield due to stress, computed as the ration of difference between  $Y_i$  and  $Y_d$  to  $Y_i$ :  $RR = AR/Y_i$ .
- DI Drought intensity index:  $(y_i - y_d)/y_i$ , where  $y_d$  and  $y_i$  are the mean yields over all genotypes evaluated under dry and well irrigated conditions, respectively. DI ranges between 0 and 1 and the larger the value of it, the more severe is the stress intensity.
- SSI Stress susceptibility index of Fischer and Maurer (1978), expressed as:  $SSI = RR/DI$ .  
Values of  $SSI < 1$  indicate below-average drought susceptibility (drought tolerance above average),  $SSI = 1$  indicate average reaction.  
Values of  $SSI > 1$  indicate above drought susceptibility (drought tolerance below average).
- GMP Geometric mean of productivity, expressed as  $(Y_d \times Y_i)^{0.5}$  by (Fernandez 1993).
- STI Stress tolerance index defined as:  $STI = Y_d \times Y_i / (y_i)^2$  (Fernandez 1993).

### Results and discussion

In this paper all results were analyzed in terms of means per local variety (over the 9 populations). Comparison of the two treatments for water content (WC) in both seasons indicates that for the 10 varieties water deficit started 2 mo after plant emergence (first WC measurement) which coincided with the beginning of the flowering of the early varieties (Figure 1). All genotypes tested expressed a reduction of WC under stress at all three stages. This reduction increased steadily with time overall with the stress intensity. Differences among local varieties for WC reduction were not significant 2 mo after sampling. However at months 3 and 4 (measurements 2 and 3), these differences were highly significant (Figure 2).

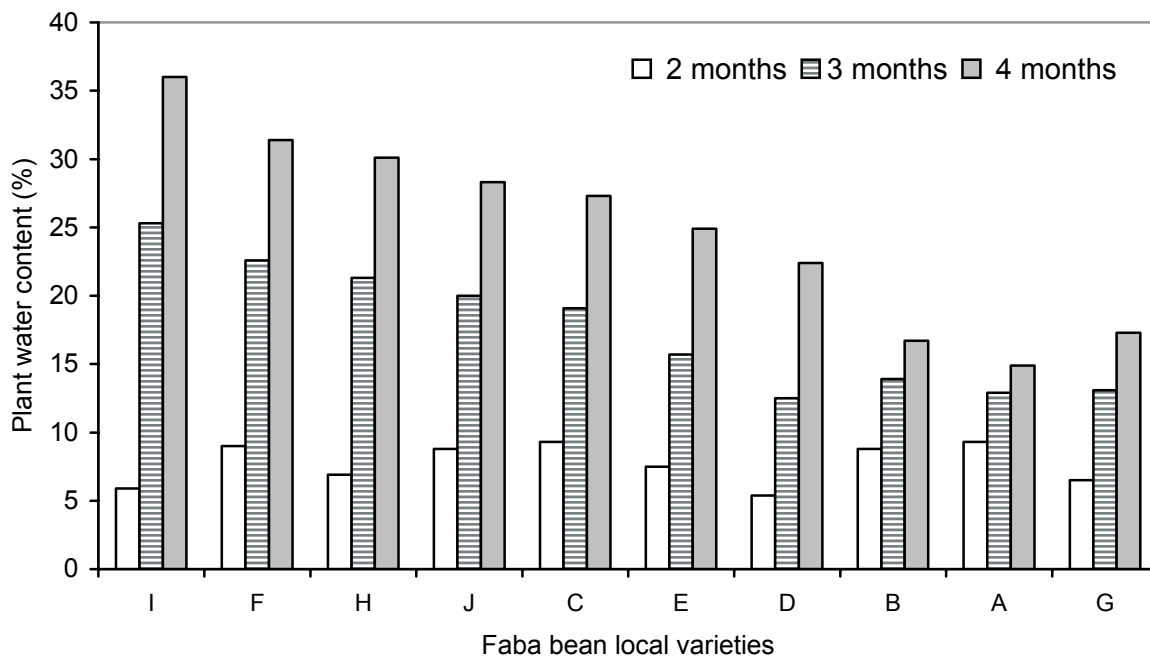
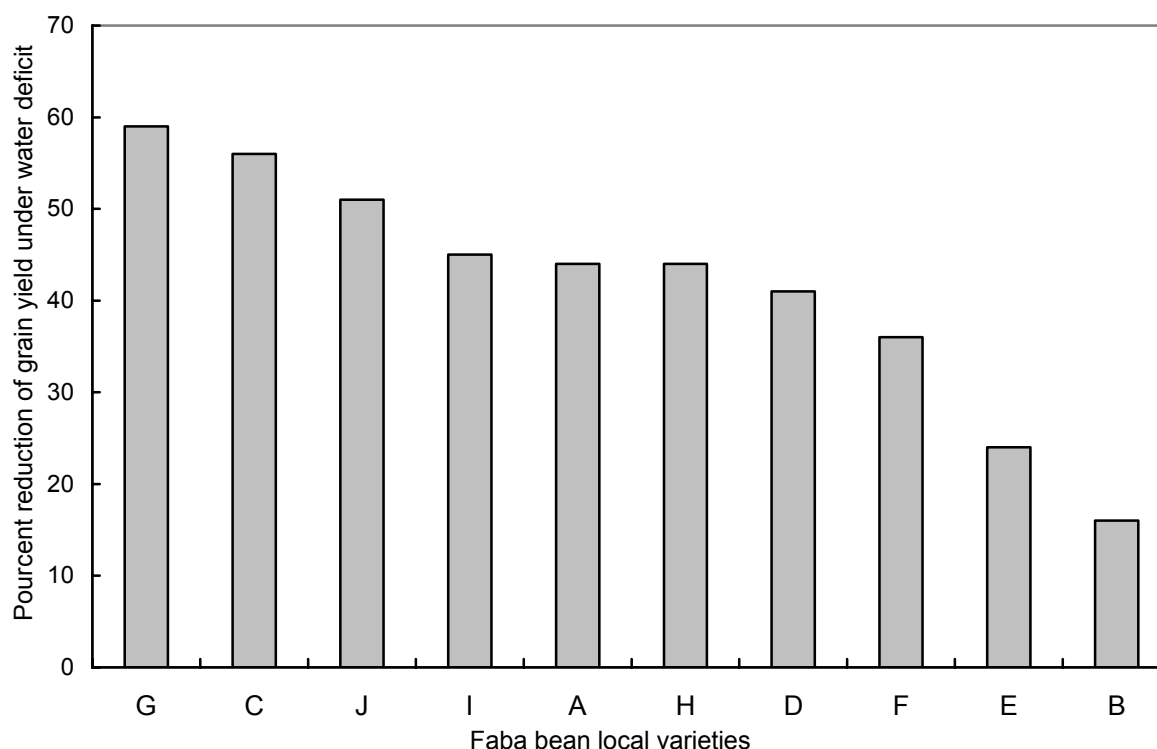


Figure 2. Ranking of faba bean local varieties based on average percent reduction of plant water content as result of water deficit at 3 different plant ages (2, 3 and 4 months).

Analysis of variance revealed that differences for plant biomass, grain yield and yield-components were highly significant among the local varieties and between the two water regimes. The interaction local variety  $\times$  water regime was also highly significant indicating that ranking of genotypes varies under the two water treatments. The local varieties are significantly different for grain yield reduction under water deficit (Figure 3). The effect of irrigation is independent of the production level. Varieties B and E expressed the least reduction (15% and 22.5% respectively). All other varieties underwent more than 35% yield reduction. Varieties C, G and J were the most affected by water deficit as their yield was reduced by more than 50% under dry treatment compared with irrigated treatment.



**Figure 3. Effect of water stress on grain yield of 10 local varieties of faba bean evaluated under 2 water regimes in the field.**

Table 1 presents the computation of drought reaction indices for the 10 local varieties as mean over accessions. The value of drought intensity index (DI) of 0.42 indicates that water stress was severe, reducing grain yield by 42% on average. Water stress effect varied significantly among genotypes (Table 1).

The possibility of combining in the same genotype drought tolerance and a high yield potential has been a subject of controversy (Ceccarelli et al. 1992). The analysis of correlation of drought-tolerance parameters with the yield under the two conditions ( $Y_i$  and  $Y_d$ ) shows that according to the nature (positive or negative) of this association these parameters can be arranged in two types. In Table 1,  $Y_d/Y_i$ , RR and SSI are significantly negatively correlated to yield ( $Y_i$  and  $Y_d$ ). These parameters define absolute drought tolerance. Similar results were reported from several studies (Fischer and Maurer 1978; Rosielle and Hamblin 1981; Schneider et al. 1997; Abdelmula and Link 1998). GMP and STI parameters expressed a positive tight relation with  $Y_i$  and  $Y_d$ . Enhancing these parameters increases yield under water stress as well as under favourable conditions. Hence as opposed to the first type these parameters describe productivity and drought tolerance simultaneously.

**Table 1. Indices of assessment of reaction to stress (susceptibility or resistance).**

| Local varieties     | Yd/Yi | AR    | RR    | SSI   | GMP   | STI  | Yi    | Yd    |
|---------------------|-------|-------|-------|-------|-------|------|-------|-------|
| A                   | 55.93 | 13.00 | 0.44  | 1.05  | 22.06 | 1.10 | 29.50 | 16.50 |
| B                   | 84.10 | 3.10  | 0.16  | 0.38  | 17.88 | 0.73 | 19.50 | 16.40 |
| C                   | 44.38 | 9.40  | 0.56  | 1.32  | 11.26 | 0.29 | 16.90 | 7.50  |
| D                   | 58.63 | 10.30 | 0.41  | 0.98  | 19.07 | 0.83 | 24.90 | 14.60 |
| E                   | 76.47 | 4.80  | 0.24  | 0.56  | 17.84 | 0.72 | 20.40 | 15.60 |
| F                   | 63.64 | 6.00  | 0.36  | 0.87  | 13.16 | 0.39 | 16.50 | 10.50 |
| G                   | 41.38 | 17.00 | 0.59  | 1.40  | 18.65 | 0.79 | 29.00 | 12.00 |
| H                   | 56.37 | 11.30 | 0.44  | 1.04  | 19.45 | 0.86 | 25.90 | 14.60 |
| I                   | 54.63 | 4.90  | 0.45  | 1.08  | 7.98  | 0.14 | 10.80 | 5.90  |
| J                   | 49.09 | 8.40  | 0.51  | 1.21  | 11.56 | 0.30 | 16.50 | 8.10  |
| Mean                | 58.46 | 8.82  | 0.42  | 0.99  | 15.89 | 0.62 | 20.99 | 12.17 |
| Minimum             | 41.38 | 3.10  | 0.16  | 0.38  | 7.98  | 0.14 | 10.80 | 5.90  |
| Maximum             | 84.10 | 17.00 | 0.59  | 1.40  | 22.06 | 1.10 | 29.50 | 16.50 |
| Correlation with Yi | -0.21 | 0.76  | -0.11 | 0.21  | 0.94  | 0.91 | 1.00  | 0.74  |
| Correlation with Yd | 0.61  | 0.14  | -0.91 | -0.61 | 0.96  | 0.91 | 0.74  | 1.00  |

When adopted to rank and compared with different local genotypes these indices should be used in conjunction with the yield value. The SSI index combining AR and RR can be used to classify the 10 local varieties in three categories as follows:

- SSI <1 indicating drought tolerance above average (Local variety B, E, F).
- SSI =1 Average drought tolerance (Local variety A, D, H, I).
- SSI >1 Drought tolerance below average (Local variety C, G, J).

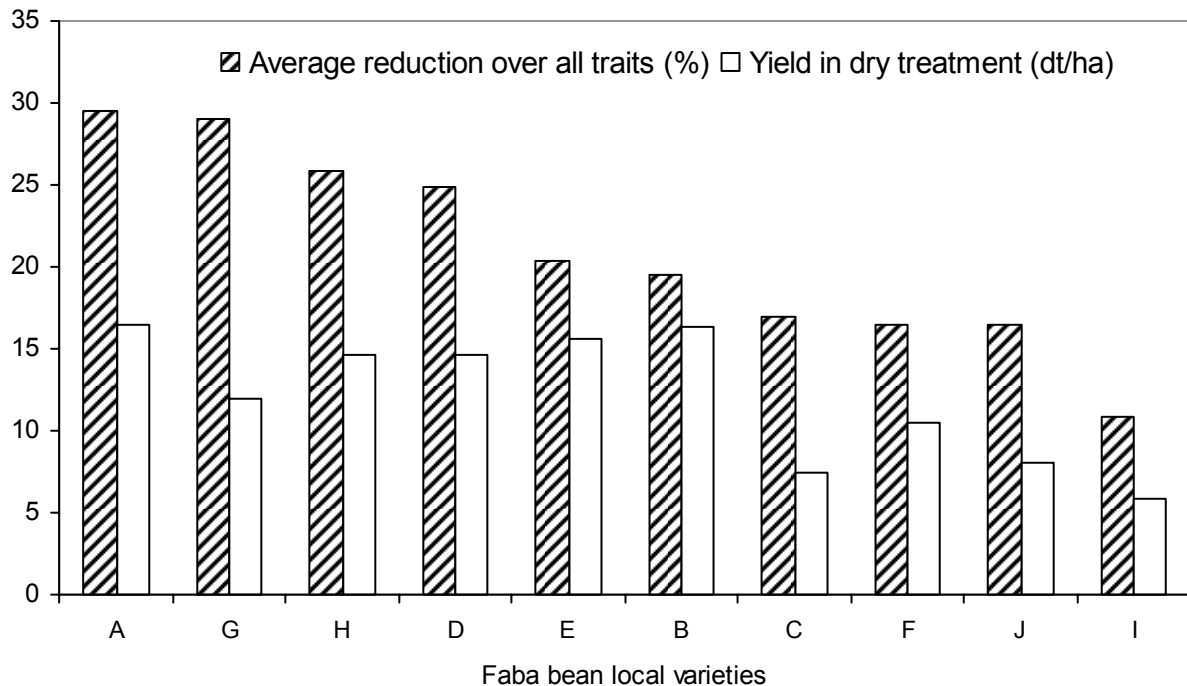
Based on this parameter and on yield (Yi and Yd) each of these categories may be subdivided into two classes according to whether yield potential is above or below average yield (Table 2). Similar classification may be applied based on other parameters positively associated to yield such as Yd/Yi. Figure 4 illustrates this classification of local varieties and specifies the level of yield under dry conditions (Yd).

The most desirable varieties are those of class 1.1 (B and E) which combine good drought tolerance and good production potential.

**Table 2. Classes of local varieties according to drought indices and production potential.**

| SSI | Reaction                        | (Yd+Yi)/2      | Genotypes | Average yield potential  | Class |
|-----|---------------------------------|----------------|-----------|--------------------------|-------|
| <1  | Drought tolerance above average | > mean (16.58) | B, E      | Good in both environment | 1.1   |
|     |                                 | < mean         | F         | Low                      | 1.2   |
| = 1 | Average drought tolerance       | > mean (16.58) | A, D, H,  | Good in both environment | 2.1   |
|     |                                 | < mean         | I         | Low                      | 2.2   |
| > 1 | Drought tolerance below average | > mean (16.58) | G         | Good in both environment | 3.1   |
|     |                                 | < mean         | C, J      | Low                      | 3.2   |

The close connection between the values of the yield in the irrigated and dry treatments ( $R = 0.74$ ) is partly explained by broad genetic variability within this collection of local populations. However, this correlation did not explain the genotype  $\times$  environment ( $G \times E$ ) interaction which was highly significant. Schneider et al. (1997) found similar significant



**Figure 4. Ranking of faba bean local varieties based on average percent reduction of trait values as result of water deficit and yield under dry treatment.**

interactions in bean. Similar positive correlations between the yield under water-stressed and favourable environments were reported by Singh (1995), Schneider et al. (1997) and Ramirez-Vailejo and Kelly (1998) in common bean, Fernandez (1993) in cowpea, and Ceccarelli (1987) in barley. However, the degree of the correlation strongly depends on the intensity of the stress and it decreases under the more severe conditions of stress (Fernandez 1993).

Whereas in wild plant species the drought tolerance is defined as survival under dry conditions, in the crop species it is defined in terms of productivity (Passioura 1983). Productivity represents average yield in the water-stressed and favourable environments. All measurements of traits associated with faba bean growth and yield and derived parameters to assess the productivity potential, allowed effective classification of the 10 local varieties analyzed. The drought tolerance indices allowed us to synthesise these parameters and rank the plant material.

#### References

- Abdelmula, A.A. and W. Link. 1998. Evaluation of drought tolerance in faba bean (*Vicia faba* L.). Pp. 54-55 in Proceeding of EUCARPIA Symposium on Breeding of Oil and Protein Crops, 1-4 April 1998, Pontevedra, Spain.
- Alami, A. 1995. Contribution à l'étude du germplasm local de fève et réponse à la sélection récurrente. Mémoire de fin d'étude. Institut Agronomique et vétérinaire Hassan II, Rabat, Morocco.
- Berrada, M. 1995. Evaluation et exploitation des ressources génétiques de *Vicia faba* L. Mémoire de fin d'étude. Institut Agronomique et vétérinaire Hassan II, Rabat, Morocco.
- Blum, A. 1983. Genetic and physiological relationships in plant breeding for drought resistance. *Water Manage.* 7:195-205.
- Blum, A. 1988. *Plant Breeding for Stress Environments*. CRC Press Inc., Boca Raton, Florida, USA.
- Ceccarelli, S. 1987. Yield potential and drought tolerance of segregating populations of barley in contrasting environments. *Euphytica* 36: 265-273.

- Ceccarelli, S. and S. Grando. 1996. Drought as a challenge for the breeder. *Plant Growth Regulation* 20:149–155.
- Ceccarelli, S., S. Grando and J. Hamblin. 1992. Relationship between barley grain yield measured in low- and high-yielding environments. *Euphytica* 64:49–58.
- Dantuma, G. and C. Grashoff. 1984. Vegetative and reproductive growth of faba bean (*Vicia faba* L.) as influenced by water supply. Pp. 61–69 in *Vicia faba: Agronomy, physiology, and breeding* (P.D. Hebblethwaite, T.C.K. Dawkins, H.C. Heath and G. Lockwood, eds.). Martinus Nijhoff Dr. W. Junk Publishers, The Hague.
- Day, W. and B.I. Legg. 1983. Water relations and irrigation response. Pp. 217–232 in *The Faba Bean (Vicia faba L.), a basis for improvement* (P.D. Hebblethwaite, ed.). Butterworths, London.
- Farah, S.M. 1981. An examination of the effects of water stress on leaf growth of crops field beans. 1. Crop growth and yield. *Journal of Agricultural Science (Cambridge)* 96:327–336.
- Fernandez, G.C.I. 1993. Effective selection criteria for assessing plant stress tolerance. Pp. 257–270 in *Adaptation of Food Crops to Temperature and Water Stress* (C.G. Kuo, ed.). AVRDC, Shanhua, Taiwan.
- Fischer, R.A. and R. Maurer. 1978. Drought resistance in spring wheat. 1. Grain yield responses. *Australian Journal of Agricultural Research* 29:897–912.
- French, B.K. and B.J. Legg. 1979. Rothamstead irrigation 1946–1976. *Journal of Agricultural Science (Cambridge)* 92:15–37.
- Grashoff, C. 1990. Effect of pattern of water supply on *Vicia faba* L. I. Dry matter partitioning and yield variability. *Netherlands Journal of Agricultural Science* 38:21–44.
- Hall, A.E., T.J. Close and E.A. Bray. 1993. Is dehydration tolerance relevant to genotypic differences in leaf senescence and crop adaptation to dry environments? *The American Soc. Plant Pathologists*, Rockville, MD.
- Hebblethwaite, P.D., J. Ingram, R.K. Scott and J. Elliot. 1977. Some factors influencing yield variation of faba beans (*Vicia faba* L.). Pp. 20–27 in *Proceedings on production, processing and utilization of the field bean (Vicia faba L.)* (R. Thompson, ed.). Bull. No. 15, Scottish Horticultural Research Institute, Invergowrie, UK.
- Langer, I., K.J. Frey and T. Bailey. 1979. Associations among productivity, production response, and stability indexes in oat varieties. *Euphytica* 28:17–24.
- Lazrak, 1995. Evaluation de la variabilité génétique du germplasm marocain de fève et de féverole. Mémoire de fin d'études. Institut Agronomique et vétérinaire Hassan II, Rabat, Morocco.
- Mehdi, S. 1994. Evaluation et exploitation en sélection des populations locales et étrangères de fève et de féverole. Mémoire de fin d'études. Institut Agronomique et vétérinaire Hassan II, Rabat, Morocco.
- Passioura, J.B. 1983. Roots and drought resistance. *Agricultural Water Management* 7:265–280.
- Peterson, C.J., J.M. Moffatt and J.R. Erickson. 1997. Yield stability of hybrid vs. pureline hard winter wheats in regional performance trials. *Crop Science* 37:116–120.
- Ramirez-Vallejo, P. and J.D. Kelly. 1998. Traits related to drought resistance in common bean. *Euphytica* 99:127–136.
- Rosielle, A.A. and J. Hamblin. 1981. Theoretical aspects of selection for yield in stress and non-stress environments. *Crop Science* 21:943–946.
- Sadiki, M. 1990. Germplasm development and breeding for improved nitrogen fixation of faba bean in Morocco. PhD Thesis.
- Sadiki, M. and D.I. Jarvis. 2005. Développement de bases pour soutenir la gestion à la ferme de la diversité génétique des cultures dans les agro écosystèmes au Maroc. Les Actes du BRG, 1 (2003) 347-357. BRG.
- Saxena, M.C., S.N. Silim and M.V. Murinda. 1986. Effect of moisture supply and fertilizer application on the yield build-up of some contrasting faba bean genotypes. *Vortr. Pflanzenzüchtg.* II:40–48.
- Schneider, K.A., R. Rosales-Sema, F. Ibarra-Perez, B. Cazares-Enriquez, J.A. Acosta-Gallegos, P. Ramirez-Vallejo, N. Wassimi and J.D. Kelly. 1997. Improving common bean performance under drought stress. *Crop Science* 37:43–50.
- Singh, S.P. 1995. Selection for water-stress tolerance in interracial populations of common bean. *Crop Science* 35:118–124.
- Stelling, D., E. Ebmeyer and W. Link. 1994. Yield stability in faba bean, *Vicia faba* L. 2. Effects of heterozygosity and heterogeneity. *Plant Breeding* 112:30–39.

## **Management of the agrobiodiversity under the clinal variation of rainfall pattern in Burkina Faso: the example of okra drought resistance**

*Mahamadou Sawadogo, Didier Balma, Leopold Some, Carlo Fadda and Devra Jarvis*

### **Introduction**

Burkina Faso is a land-locked country of 274 000 km<sup>2</sup> with a total population of ca. 12 million people. The agricultural sector dominates the economy of the country in terms of employment, income and export opportunities. Most of the agricultural production can be considered subsistence agriculture, non-irrigated and highly dependent on the rain, which is very variable.

The country has a high latitudinal diversity in its rainfall patterns, from the north with less than 400 mm/year to the south with more than 1000 mm/year. This pattern has changed over the past 50 years with the lowest isohyets in the north of the country shifting from 500 to 400 mm/year and the highest from 1200 to 1100 mm/year in the south, thus showing a clear overall shift of the isohyets from north to south. Figure 1 shows the shifting of the isohyets in the past 50 years. The impact is a reduction of 100 mm/year of rain throughout the country (Some et al. 2004).

Besides the overall reduced amount of rain it is worthwhile to note that in the dry area of Dori in the Sahelian region there has been a reduced amount of rain in the month of May. Between 1960 and 1979 on only one occasion was there no rain in May, whereas in the next 20 years (between 1980 and 1999) there were five occasions. This means that farmers have to postpone the sowing season from April/May until June and make it shorter. In the Po Region in the isohyets of 900 mm/year the variability is much less and therefore no significant changes are required in the sowing period or occur in the growth duration.

For the temperature, there has been an increase of about 1°C over the past 50 years in Dori region while in Po there was almost no difference (Some et al. 2004).

This pattern of rainfall and temperature affects the way farmers in different regions manage their crops and the number of crops available to farmers. This is shown in Figure 1A, where the different regions are indicated together with the main crops grown. For example, crops such as cassava, rice, yam and soy are only grown in the most southern regions and only cowpea and pearl millet are available in the most northern part of the country.

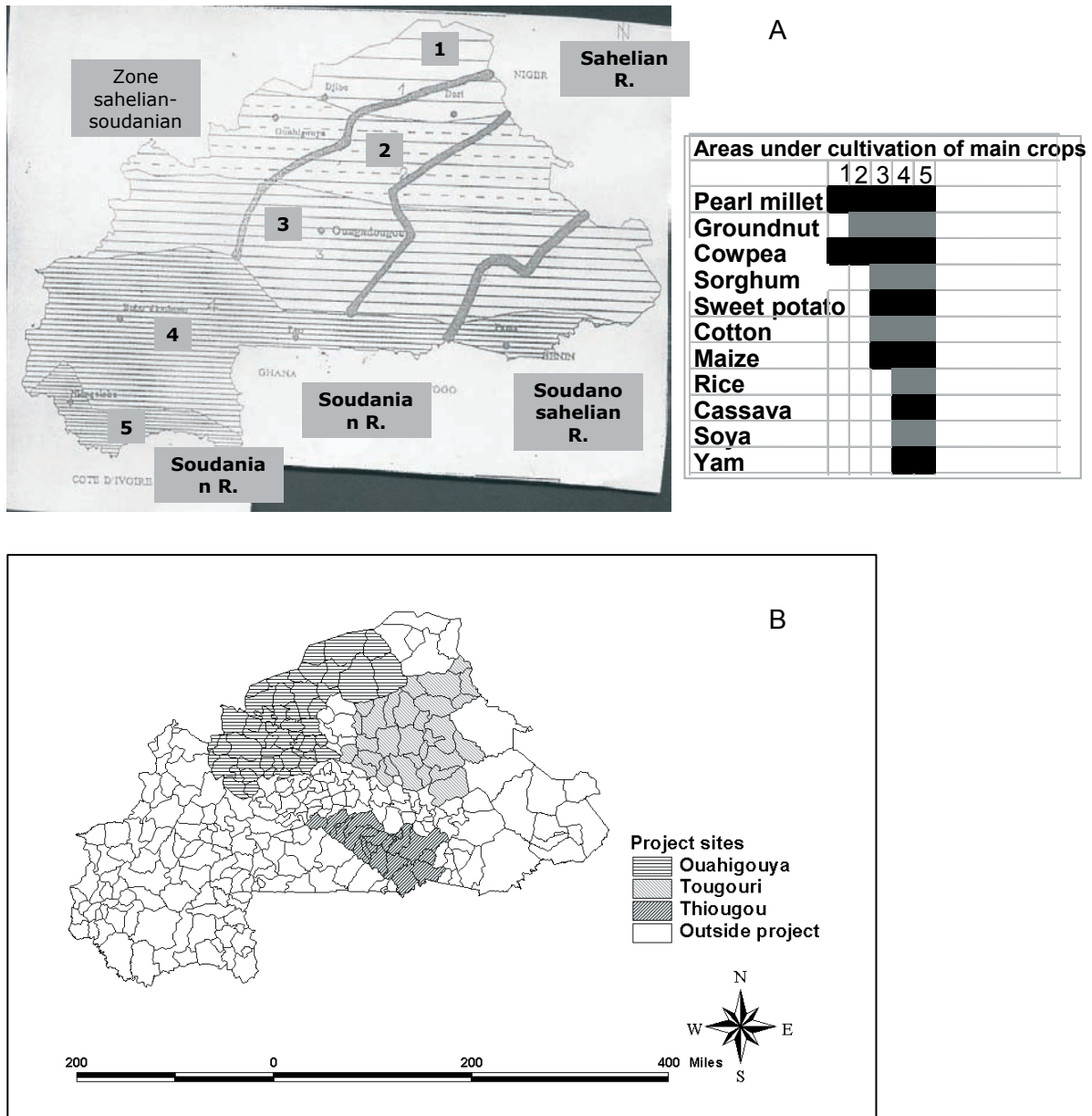
As one can expect, the differences between the north and south of the country are quite large and, particularly in the north, resistance to drought is a key factor for farmers' livelihood.

### **Methods**

The project has been implemented in three ecoregions—northeast and northwest in the central part of the country—with isohyets ranging from 400 to 900 mm/year, as shown in Figure 1B. In each region, six villages were selected based on their reliance on traditional varieties for their livelihood (i.e. villages which have received very little exposure to improved varieties), the presence of NGOs and other development agencies, and the need to improve livelihood through better use of the available genetic material (see Balma et al. 2004 for a complete report on the project).

The target crops are cowpea, sorghum, pearl millet, groundnut and okra. Sorghum and pearl millet have been selected as the major crops, groundnut and cowpea are cash crops, and okra is important because it is a cash crop managed by women, thus being an important economic factor for women (Dossou et al. 2004).





**Figure 1. A: Main agroecological regions and main crops cultivated in each; B: Project sites.**

As a first step, drought resistance on okra was tested on-station in order to understand the performances of different varieties. This crop is typically managed by women. Its genetic diversity has received very little attention as far as West Africa is concerned (Hamon 1983). Few studies are available on agromorphological characteristics (Fondio and Kouame 1999) or on its interaction with the environment (Ariyo and Ayo 2000). To understand these, 70 ecotypes were collected in eight different agroecological regions of Burkina and evaluated during the rainy season of 2002 (for full details on this study see Sawadogo et al. 2006).

The trials were implemented at Ouagadougou University. Three randomized blocks with three repetitions were used. Every ecotype was sown in six (6) pots: three of them served as checks (watered twice each day) against the three others, called the tests (whose watering was interrupted at the appearance of 50% of floral buttons or 50% flowering). A total of 18 plants for each variety was evaluated for 12 variable traits:

- 50% of flowering plants (50% F), number of plants harvested (NPR), plant height at maturity (LP), main fruit length (LF), main fruit width (DF), diameter of plant stem (DP)
- 6 other variable traits related to yield including the mean weight of fruit per plant (PF), total number of fruits harvested per plant (NF)
- 4 variable traits related to drought resistance: water content compared with dry biomass (TES), water content compared with biomass (TEF), relative water content (RWC) and permeability of the membrane (PM).

Simple variance analysis was conducted with one-way ANOVA (SAS and STAT-ITCF). The links between variable traits were proved using total correlation calculation.

As a second step, the diversity for okra and other crops was measured at six villages in each of the different ecoregions, using the Shannon-Weaver and Simpson indices of diversity. Different methods were used to collect the information, including individual interviews, key informant interviews and group semi-structured interviews. This allowed collecting of information on units of diversity and their characteristic traits, including morphological and physiological traits, management practices, the importance of different varieties for their livelihood with respect to drought and other environmental factors (Sawadogo et al. 2004c), and how decisions are made on what to plant (prediction of the rainfall) based on natural signs (Sawadogo et al. 2004a).

The significance of the differences in diversity for each crop at each site was tested using a one-way ANOVA and this provided a clear understanding of diversity at each site.

### **Results**

#### **Resistance of okra to drought**

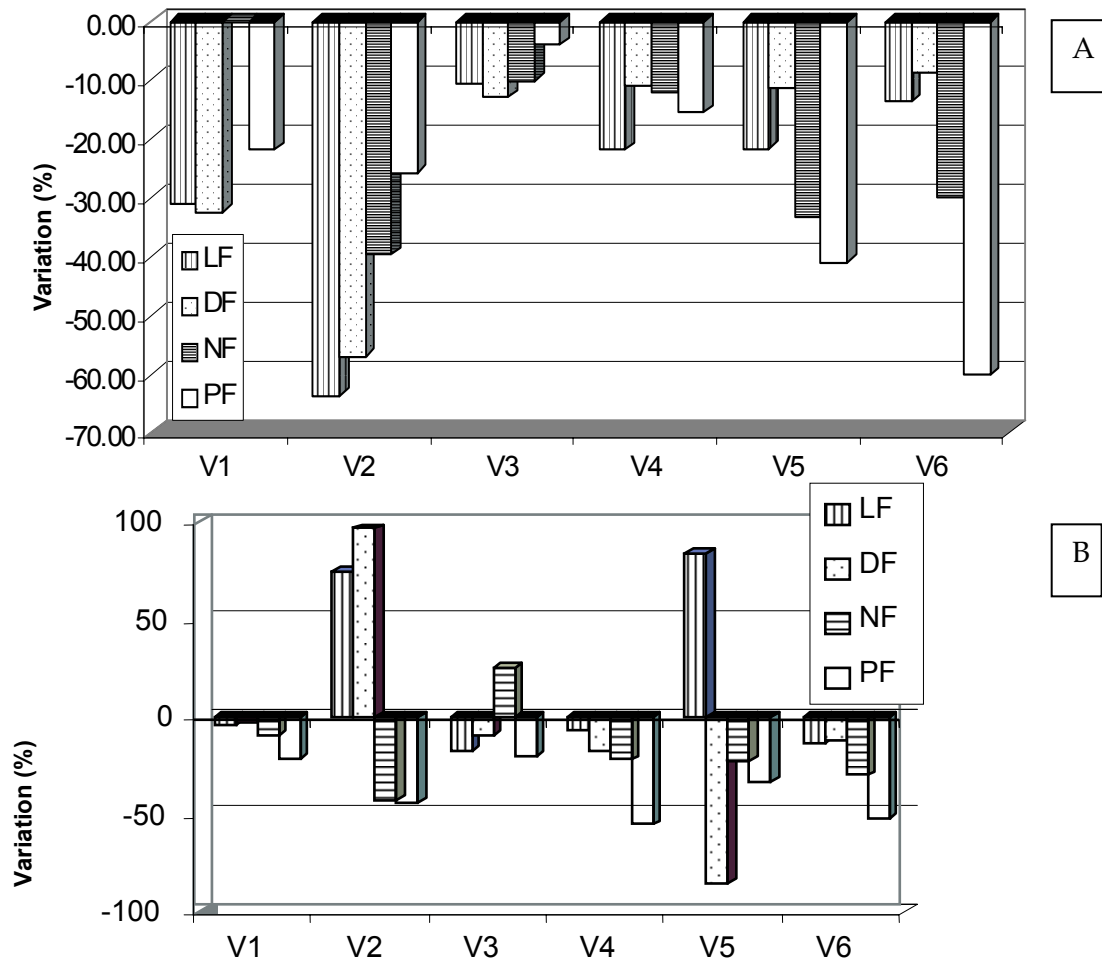
Resistance of okra to drought shows a high diversity among varieties. In general when a water shortage occurs during budding there is a reduction in fruit length, fruit weight, number of fruits produced and fruit width to different extents. This is more accentuated for V2 while V3 and V4 have the minimum reduction in yield (Figure 2A). When water stress was applied during the flowering of the plants the response was much more variable (Figure 2B): ecotypes V1, V4 and V6 had a reduction in yield, while V2 and V5 had a significant increase in fruit size and V3 had an increased number of fruits and a reduction for the other parameters.

There was an important decrease in resistance when the water shortage occurred at budding. However, it is very significant that the membrane permeability increased, particularly for V1, V2, V5 and V6. This phenomenon could be the origin of cell metabolism problems at the moment of watering (Hopkins 2003). The same pattern was observed when the stress was applied at flowering, except that V3 had a strong reduction in the permeability of the membrane. The different ecotypes showed different responses depending on the time at which stress was applied. Varieties V1, V3 and V4 were the more resistant when stress was applied at budding, while V5 and V6 were the least resistant. When the stress was applied at flowering, V1 and V3 were the more resistant while V2, V4 and V6 were the more susceptible.

Stem growth also showed a reduction in growth pattern. In general, okra shows two patterns of growth: some varieties grow very quickly and flowering occurs during this accelerated growth (V3, V4, V5 and V6m), whereas type II okra flowers when the accelerated growth is complete (V1 and V2).

In the first group, water stress had the effect of accelerating the flowering time (compared with the control group), thus reducing the cycle duration, while for the second group the effect was a delay in flowering and a longer cycle.

Overall, there were different responses to water stress among different ecotypes.



**Figure 2. Differences in the fruit length (LF), fruit diameter (DF), number of fruits produced (NF), and fruit weight (PF) when water stress is applied during budding (A) and flowering (B).**

### The observed pattern of variation

An important result from the interview with the farmers is their perception of the environment because their decision on what to plant is based on their perception of what will happen in the next season in terms of rainfall. Although they always plant a high number of varieties to protect against risks of crop failure, their perception of whether the next season will be very dry or not will let them decide which varieties should be considered as a priority and when to plant. Signals are taken from plants (e.g. maturity of fruits or appearance of leaves in some natural trees), birds (time of egg deposition for guinea fowls or storks returning from Europe), other animals (lizards shedding skin or toads moving from the ponds to the bush and singing frequently), stars and weather (appearance of some constellations, wind blowing from the east, increased temperature, appearance of clouds), and rituals used to predict weather by rainmakers.

High Simpson indices are found as shown in Table 1. Overall evenness is significant for all crops but okra. When looking at pairwise comparisons, however, sites 1 and 2 are more similar to each other than to site 3. This is reflected in that the differences between the first two sites are only significant for cowpea. Differences between site 3 and the other two sites, in contrast, are always significant except for okra. In general, farmers from site 3 keep a significantly higher diversity of groundnut and sorghum and a significantly lower diversity of cowpea and pearl millet, while farmers from site 1 maintain a higher diversity of pearl millet and cowpea.

The correlation between richness and Simpson index was always higher than 0.85. Interestingly, when the total richness was standardized by the total diversity available at each site, the pattern was quite different (Table 1). For cowpea the differences were not significant, with farmers growing between 15 and 20% of the total number of varieties available; for groundnut at site 1, farmers manage a smaller proportion of the available diversity (ca. 20% vs. almost 30% at the other 2 sites); for okra, differences were always significant with farmers at site 2 managing the highest proportion of the total available diversity (ca. 40%) and farmers of site 1 managing the smallest proportion (less than 20%). This is also reflected in low divergence values between farms. For cereals, the biggest proportion of pearl millet was managed in site 2 (more than 25% of the total diversity) which shows significant differences from the other 2 sites (about 17% of total diversity grown by farmers). In sorghum the differences were significant among all sites, with site 1 managing the smaller proportion of available richness (14%) and site 2 the highest proportion (25%).

**Table 1. Shannon-Weaver and Simpson indices of proportion of varieties grown over the total and significance between sites.**

| Crop         | Site | Shannon-Weaver index | Mean of Simpson index | Significance of pairwise comparison | Mean of proportion of grown vars./ total available varieties | Significance of pairwise comparison |
|--------------|------|----------------------|-----------------------|-------------------------------------|--|-------------------------------------|
| Cow pea      | 1    | 1.12                 | 0.4331332             | 1-2 **                              | 0.1706349  | 1-2 NS                              |
|              | 2    | 0.59                 | 0.1994399             | 1-3 **                              | 0.1777778  | 1-3 NS                              |
|              | 3    | 0.53                 | 0.2560222             | 2-3 NS                              | 0.1944444  | 2-3 NS                              |
| Groundnut    | 1    | 0.79                 | 0.3505772             | 1-2 NS                              | 0.1944444  | 1-2 **                              |
|              | 2    | 0.67                 | 0.2621063             | 1-3 ***                             | 0.2777778  | 1-3 ***                             |
|              | 3    | 1.00                 | 0.5617884             | 2-3 ***                             | 0.2944444  | 2-3 NS                              |
| Okra         | 1    | 0.92                 | 0.4461764             | 1-2 NS                              | 0.1805556  | 1-2 ***                             |
|              | 2    | 0.65                 | 0.5315978             | 1-3 NS                              | 0.3777778  | 1-3 ***                             |
|              | 3    | 0.91                 | 0.465224              | 2-3 NS                              | 0.2777778  | 2-3 ***                             |
| Pearl millet | 1    | 1.16                 | 0.553405              | 1-2 NS                              | 0.1633987  | 1-2 ***                             |
|              | 2    | 1.06                 | 0.509882              | 1-3 ***                             | 0.2638889  | 1-3 NS                              |
|              | 3    | 0.85                 | 0.3358921             | 2-3 **                              | 0.1761364  | 2-3 ***                             |
| Sorghum      | 1    | 2.00                 | 0.6742812             | 1-2 NS                              | 0.1399177  | 1-2 ***                             |
|              | 2    | 1.57                 | 0.645346              | 1-3 ***                             | 0.2541667  | 1-3 **                              |
|              | 3    | 1.65                 | 0.7537091             | 2-3 ***                             | 0.1845238  | 2-3 ***                             |

### Conclusions

The results of studies on drought resistance of okra show that the response of different ecotypes is very diverse and that only a few of them are resistant to this abiotic stress, i.e. V1, V3 and V4. In fact, every year there is a period of drought in the selected agroecosystems, which forces farmers to select varieties that have a fast growth cycle (between 43 and 60 days). However, the response to the stress is not the same for all ecotypes even though they can all survive a stress period ranging from 10 to 14 days. In general, water stress occurring at the budding stage is much more damaging than stress occurring at flowering as the former results in a significant reduction in all production characteristics (number of fruits, weight and length of the fruit, etc.). For water stress, ecotypes V3 and V4 cope the best and should be recommending for planting at the very beginning of the season, which is when a drought is more likely to occur. When water stress occurs at flowering the response is even more diverse. In general the growth pattern of okra is very fast between budding and flowering, with a slow down after flowering. In this respect, two main groups are recognized: those flowering when the growth rate is already reducing its pace and those flowering when the growth rate is still fast. The latter group is the one that shows a better

response to water stress, by producing early flowers. The ecotypes showing the best response to stress are V1, V3 and V4 and should be recommended to the local communities.

Therefore, if only drought resistance is selected as a character, one should expect an overall reduction in diversity. However, the picture is quite different. The results illustrated above offer an opportunity to discuss farmers' management of different crops under different environmental conditions, and the resistance of different okra varieties under water stress, a typical situation occurring in Burkina Faso.

Farmers tend to manage a high number of varieties in order to cope with environmental stress. A shortage of rain in May may force farmers to plant both late and early maturing varieties. If the rain is not sufficient for varieties having a longer cycle, at least they will harvest from the early maturing varieties.

It is interesting to interpret the results in light of the function of the crops. For the two cereals—the subsistence crops—Simpson values tend to be very high in the three sites, but pearl millet (the main cereal in the north, at sites 1 and 2) has values higher than 0.50 in these two sites, while the total richness is higher in site 1, and thus a lower proportion of the available diversity is used by farmers. In site 2, farmers manage the same overall diversity by using a higher proportion of the total available diversity. The situation is reversed for sorghum, the Simpson index values of which are even higher than the one for pearl millet—ranging from 0.64 to 0.75—but farmers in site 3 keep more varieties. Again, the overall diversity is lower in site 2 and again farmers use the highest proportion of available diversity in order to manage the same diversity as in site 1 on their plots. The management practices and a full description of diversity are reported in Sawadogo et al. (2004b).

For the cash crops, the diversity kept on-farm is lower than for the subsistence crops except for cowpea in site 1 (the main cash crop in the area) and for groundnut in site 3 (the main and very traditional cash crop in this site). In other words, farmers tend to keep a higher diversity of their cereals and a lower diversity of the cash crop except for the main one. For okra, the level of diversity is comparable to pearl millet in all sites. Even if this is mainly a cash crop, it is managed typically by women, showing the important role played by women in keeping diversity (Dossou et al. 2004).

Although farmers do not choose their varieties only on the basis of drought resistance, these results clearly show that when only this factor is considered, only a few varieties can cope with it, thus leading to a potential reduction in overall agrobiodiversity. A balance between drought resistance and other important characters used by farmers to choose their preferred varieties must be found in order to maintain a high biodiversity.

## References

- Ariyo, O.J. and V.M.A. Ayo. 2000. Analysis of genotype  $\times$  environment interaction of okra (*Abelmoschus esculentus* L. Moench). *Journal of Genetics and Breeding* 54 (1):35–40.
- Balma, D., B. Dossou, M. Sawadogo, R.G. Zangré, J.T. Ouédraogo et D. Jarvis (eds.). 2004. La gestion de la diversité des plantes agricoles dans les agro-écosystèmes : compte-rendu des travaux de l'atelier scientifique organisé par le CNRST, l'INERA et l'Institut International des Ressources Phytogénétiques à Ouagadougou, Burkina Faso du 27–28 décembre 2002. IPGRI, Nairobi, Kenya.
- Dossou, B., D. Balma and M. Sawadogo. 2004. Le rôle et la participation des femmes dans le processus de conservation *in situ* de la diversité biologique agricole au Burkina Faso. *In* La gestion de la diversité des plantes agricoles dans les agro-écosystèmes (D. Balma, B. Dossou, M. Sawadogo, R.G. Zangré, J.T. Ouédraogo et D. Jarvis, eds.). *Compte-rendu des travaux de l'atelier scientifique organisé par le CNRST, l'INERA et l'Institut International des Ressources Phytogénétiques à Ouagadougou, Burkina Faso du 27–28 décembre 2002*. IPGRI, Nairobi, Kenya.
- Fondio, L. and C.N.G. Kouame, 1999. Sowing density, growth and yield of two okra cultivars (*Abelmoschus* spp.) in Côte d'Ivoire. *Cahiers Agriculture* 8 (5):413–415.
- Hamon, S. 1983. Discrimination de deux espèces de gombo cultivées en Côte d'Ivoire (*A. esculentus* et *A. sp.*) sur la base de leurs profils enzymatiques. ORSTOM Adiopodoumé, rapport multigraphié.
- Hopkins, W.G. 2003. Physiologie des stress. Pp. 451–473 *in* Physiologie végétale. Université d'Ontario, Ontario, Canada.

- Sawadogo, M., J. Ouedraogo, M. Belem, R.G. Zangre, J.-B. Ouedraogo and D. Balma. 2004a. Influence des facteurs écologiques et culturels sur les décisions du paysan dans la conservation *in situ* de la diversité biologique agricole. *In* La gestion de la diversité des plantes agricoles dans les agro-écosystèmes (D. Balma, B. Dossou, M. Sawadogo, R.G. Zangré, J.T. Ouédraogo et D. Jarvis, eds.). Compte-rendu des travaux de l'atelier scientifique organisé par le CNRST, l'INERA et l'Institut International des Ressources Phytogénétiques à Ouagadougou, Burkina Faso du 27–28 décembre 2002. IPGRI, Nairobi, Kenya.
- Sawadogo, M., J.T. Ouedraogo, R.G. Zangre and D. Balma. 2004b. Diversité biologique agricole et les facteurs de son maintien en milieu paysan. In : D. Balma, B. Dossou, M. Sawadogo, R.G. Zangré, J.T. Ouédraogo et D. Jarvis (eds.). *In* La gestion de la diversité des plantes agricoles dans les agro-écosystèmes (D. Balma, B. Dossou, M. Sawadogo, R.G. Zangré, J.T. Ouédraogo et D. Jarvis, eds.). Compte-rendu des travaux de l'atelier scientifique organisé par le CNRST, l'INERA et l'Institut International des Ressources Phytogénétiques à Ouagadougou, Burkina Faso du 27–28 décembre 2002. IPGRI, Nairobi, Kenya.
- Sawadogo, M., M. Ouedraogo and D. Balma. 2004c. Module de formation en sélection variétale participative/amélioration génétique participative: offrir au paysan des options et voies appropriées d'utilisation de la variabilité génétique dans le renforcement du maintien *in situ* de la diversité biologique agricole *In* La gestion de la diversité des plantes agricoles dans les agro-écosystèmes (D. Balma, B. Dossou, M. Sawadogo, R.G. Zangré, J.T. Ouédraogo et D. Jarvis, eds.). Compte-rendu des travaux de l'atelier scientifique organisé par le CNRST, l'INERA et l'Institut International des Ressources Phytogénétiques à Ouagadougou, Burkina Faso du 27–28 décembre 2002. IPGRI, Nairobi, Kenya.
- Sawadogo, M., G. Zombre and D. Balma. 2006. Expression de différents écotypes de gombo (*Abelmoschus esculentus* L.) au déficit hydrique intervenant pendant la boutonnisation et la floraison. *Biotechnol. Agron. Soc. Environ.* 2006 10 (1) :1–2.
- Some, L., T. Lodun and B. Some. 2004. Les facteurs agroécologiques dans la conservation *in situ* de la biodiversité agricole. *In* La gestion de la diversité des plantes agricoles dans les agro-écosystèmes (D. Balma, B. Dossou, M. Sawadogo, R.G. Zangré, J.T. Ouédraogo et D. Jarvis, eds.). Compte-rendu des travaux de l'atelier scientifique organisé par le CNRST, l'INERA et l'Institut International des Ressources Phytogénétiques à Ouagadougou, Burkina Faso du 27–28 décembre 2002. IPGRI, Nairobi, Kenya.

## Characterization of local maize landraces from Zimbabwe, Zambia and Malawi

*C. Magorokosho, M. Bänziger and F. J. Betrán*

### **Introduction**

Maize dominates the food economy of southern Africa, where it is by far the dominant staple crop grown by the vast majority of rural households. Per capita annual consumption of maize averages more than 100 kg in Zimbabwe, Zambia and Malawi (Aquino et al. 2001). The Portuguese introduced maize in Africa beginning in the 16<sup>th</sup> century and since then the crop has replaced sorghum and millet as the main staple in most of the continent (McCann 2005). Early maize introductions in Zimbabwe, Zambia and Malawi consisted mostly of flint and soft floury types originating mainly from Brazil and the Caribbean (McCann 2005) followed later in the 20<sup>th</sup> century by open-pollinated dent types from the USA (Weinmann 1972). Since then, local maize populations have been cultivated and subjected to natural and human selection in different environments and cultural methods in the three countries. Over this time, new landraces have been created from the original populations introduced, through smallholder farmer selection for adaptation to local conditions as well as hybridization that resulted from continuous exchange and trade between communities. Smallholder farmers in the three countries have continued to cultivate and maintain local maize populations up to today even though improved varieties have been available since the early part of the 20<sup>th</sup> century. The environments and farming systems in Zimbabwe, Zambia and Malawi where maize is grown are extremely diverse with production varying considerably between years, and showing a close dependence on rainfall and soil fertility (Aquino et al. 2001). Information about the impact of smallholder farmer selection on abiotic stress tolerance of maize is mostly lacking. Farmers' local varieties collected from marginal environments may possess some unique physiological attributes that may not be present in germplasm not exposed to abiotic stress (Blum and Sullivan 1986). The objective of this study was to assess the level of phenotypic diversity in maize landraces that have been under farmer selection for over 100 years in different agroecologies in the three countries. This paper is part of a wider study to identify potential sources of new genes for tolerance to abiotic stress in maize.

### **Methods**

#### **Collection of sample**

From June to August 2003, maize landraces were collected from smallholder farmers in Zimbabwe, Zambia and Malawi by an expedition team comprising CIMMYT and Ministry of Agriculture staff for each respective country. Smallholder areas are predominantly located in marginal areas with low rainfall coupled with poor soil fertility. Many farmers in these areas still cultivate maize landraces or local populations. The expedition team used a modification of the stratified sampling strategy recommended by Brown and Marshall (1995). The stratification involved a combination of agroecological and farming system parameters, and local knowledge by agriculture extension staff. A 1° longitude by 1° latitude grid system was superimposed on a map of the country (i.e. grid squares) and samples were collected in 150 grids across the three countries. The grids spanned all the principal maize agroecologies defined for the three countries (Hartkamp et al. 2000). In each grid, samples were collected at 1–2 randomly chosen farms per agroecology. At each farm, 2 kg of seed or an equivalent amount of maize ears was collected. This sample has been found to adequately represent most of the diversity in a maize population as recommended by Crossa (1989). Passport data

and qualitative data on the characteristics of each sample were recorded on a survey form that had been prepared beforehand.

### **Phenotypic evaluation**

A set of maize varieties comprised of 267 local landraces collected from smallholder farmers in the three southern African countries, 6 original OPVs introduced into Zimbabwe, Zambia and Malawi from the USA, 5 historically important OPVs for the region, and 16 improved varieties developed in the three countries was evaluated in a field trial during the 2003/04 season at CIMMYT Harare to determine the pattern of phenotypic diversity and classify the varieties in groups. The trial was planted on 26 November 2003 as an alpha-lattice design with two replications. Plot size was 3.375 m<sup>2</sup> and each plot consisted of 17 hills in one row 75 cm apart and 4 m long, resulting in a density of 5.3 plants/m<sup>2</sup>. Fertilizer equivalent to 170–56–24 kg/ha of N–P<sub>2</sub>O<sub>5</sub>–K<sub>2</sub>O was applied following local practices. Pests and weeds were controlled when necessary.

During the growing season, data were collected as follows: (a) on a plot basis: number of days from planting to 50% of the plants shedding pollen; number of days from planting to 50% of the plants having silks at least 1 cm long; silk colouration recorded as red or white; percentage of plants with stems inclined by more than 45° (root lodging); percentage of plants with stems broken below the main ear (stalk lodging); number of ear shoots per plant; number of plants with no ears (%); (b) on five plants taken at random within each row: tassel length (cm); number tassel branches; ear leaf length (cm); ear leaf width (cm); leaf erectness (1 = lax to 9 = erect); (c) at milk stage: plant height (cm); ear height (cm); stalk diameter (cm). Adjusted means for anthesis date, number of kernel rows per cob, 100-kernel weight, kernel length, kernel width, plant height, ear height, ear length, ear diameter, rachis diameter, cob diameter and stalk circumference were used in classifying the varieties. These characters were reported to be among the most heritable and discriminatory morphological and agronomic variables for racial classification of maize (Sanchez-Gonzalez et al. 1993). A Ward Modified Location Model (MLM) method (Franco et al. 1997) was used to perform the classification using the SAS procedure PROC CLUSTER (SAS Institute Inc. 1996). The rest of the important agronomic traits were used to describe the characteristics of each of the groups formed from the cluster analysis.

## **Results**

### **Landraces collected**

Aggregation of the collections by country and agroecology showed that maize landraces are still cultivated in all of the agroecologies found in the three countries (Table 1). Grain colour was predominantly white although other colours are found in the collection (Figure 1a). A range of different grain textures was also observed in the collection (Figure 1b). The number of kernel rows on the ears ranged from 8 to 22. Most of the landraces are maintained by the farmers because of their adaptation to marginal areas and yield stability under both biotic and abiotic stress conditions. The main factors mentioned by the farmers were tolerance to drought, early maturity, low input requirements and resistance to storage weevils. The underlying factor is obtaining a sufficient harvest in an uncertain environment.

In addition, a significant number of landraces were maintained by farmers because of their unique or better taste over commercial varieties. Better ear and grain appearance and good post-harvest processing qualities were also mentioned by the farmers as some of the reasons why they continue to grow landraces despite the advent of improved varieties. Nonetheless, a consistent part of the landraces is maintained because of seed security issues. Most of the farmers who grow landraces stated that hybrid varieties could not be successfully used as saved seed in comparison to the landraces. Most of the farmers used saved landrace seed in periods of great distress, e.g. drought years, or as a security



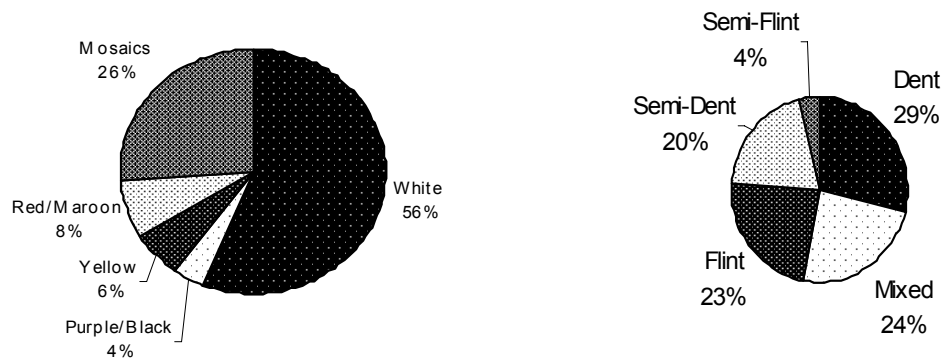
precaution. An additional reason for preserving the landraces was early maturity (drought escaping), which makes them available for food before the improved OPVs and hybrid varieties.

**Table 1. Landraces collected in different agroecologies in Zimbabwe, Zambia and Malawi.**

| Country  | Agroecology <sup>†</sup> |    |    |    |   | Total |
|----------|--------------------------|----|----|----|---|-------|
|          | A                        | B  | C  | E  | F |       |
| Zimbabwe | 22                       | 28 | 13 | 37 | 0 | 100   |
| Zambia   | 67                       | 35 | 0  | 9  | 0 | 111   |
| Malawi   | 26                       | 17 | 0  | 10 | 3 | 56    |
| Total    | 112                      | 80 | 13 | 54 | 3 | 267   |

| Agroecology | Maximum temperature (°C) | Season precipitation (mm) | Subsoil pH (water) |
|-------------|--------------------------|---------------------------|--------------------|
| A           | 24–27                    | >700                      | <5.7               |
| B           | 24–27                    | >700                      | >5.7               |
| C           | 24–30                    | <700                      |                    |
| E           | 27–30                    | >700                      | >5.7               |
| F           | >30                      | >700                      |                    |

<sup>†</sup> Characteristics of maize agroecologies where landraces were collected (Adapted from Hartkamp et al. 2000).



**Figure 1. (a) Percentage of grain colour types for the different landraces collected in Zimbabwe, Zambia and Malawi; (b) Percentage of grain texture types for the different landraces collected in Zimbabwe, Zambia and Malawi.**

### Phenotypic evaluation

Analyses of variance revealed that almost all of vegetative, ear and kernel related traits had significant variation among the varieties tested. Three distinct non-overlapping groups were obtained using cluster analysis for the 294 varieties (Figure 2). Group 1 was composed entirely of local landraces and characterized by the lowest grain yields among the three groups (Table 2). Group 2 was composed mostly of local landraces that have phenotypic characteristics similar to the OPV Hickory King, historically important OPVs from Zimbabwe (Salisbury White, Southern Cross), and Hickory King from USA. This group was characterized by intermediate grain yields. Group 3 had the highest average grain yield and was comprised of improved varieties from the seed companies in the three countries, most of the introduced OPVs from the USA, OPVs from CIMMYT and the Zambia National Program, and a few local landraces.

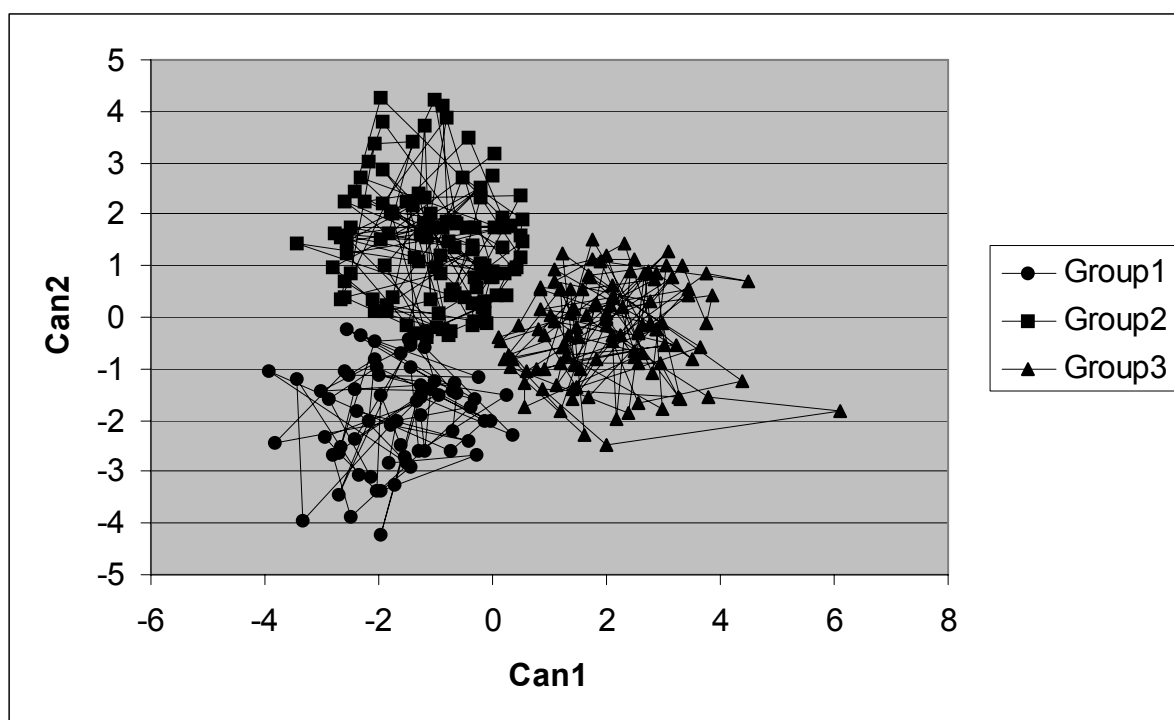


Figure 2. Plots of the first (Can1) and second (Can2) canonical variables of the 294 maize varieties grouped in 3 clusters based on key morphological and agronomic traits.

Table 2. Key characteristics of the three groups formed from cluster analysis of the 294 varieties evaluated for agromorphological diversity.

| Characteristic                   | Group                  |   |   |
|----------------------------------|------------------------|---|---|
|                                  | 1                      | 2   | 3                                       |
| n                                | 68                     | 113   | 113                                     |
| 50% Anthesis (dap <sup>†</sup> ) | 75                     | 75  | 72                                      |
| Grain yield (Mg/ha)              | 3.395                  | 4.388   | 4.932                                   |
| Ears per plant                   | 0.76                   | 0.77  | 0.88                                    |
| 100-kernel weight (g)            | 41.4                   | 50.9  | 38.2                                    |
| Kernel row number                | 11                     | 10  | 13                                      |
| Group composition                | Mostly local landraces | Historic OPVs from Southern Africa, HK type landraces, USA HK | Improved cvs, USA OPVs, a few landraces |

<sup>†</sup> dap = days after planting.

### Discussion

The present study showed that maize landraces collected by the expedition team maintain considerable genetic variation in morphological and agronomic traits. Smallholder farmers in the three countries traditionally collect seeds from those plants with better growth forms with desirable ear and kernel characteristics under their environment. Each year the selection of seeds is limited to a few chosen individuals and seeds are bulked and kept for the next planting. Thus the local varieties grown in the various countries have adapted to local conditions and farmers' practices, and represent unique sources of diversity. Many useful traits have developed in these areas following natural and farmer's selection over the years. These landraces could offer new alleles for abiotic stress tolerance in maize. Although local varieties have not been used extensively by breeders because of their other undesirable

agronomic traits, they can serve as sources of new desirable traits to enhance performance of germplasm under abiotic stresses such as drought, low soil fertility and acid soils (Beck et al. 1997).

The clustering pattern based on morphological and agronomic traits points out that farmers' adaptive selection occurring in areas where the landraces grown in different agroecologies of the three countries facilitated the accessions to maintain their distinct identities. The fact that most landraces clustered separately from improved varieties (Figure 2) indicates that maize breeders in the region have been selecting for different morpho-agronomic traits as compared to smallholder farmers (Table 2). Thus within the landraces there exists a vast amount of variation, much of which is not present in advanced breeding lines and improved varieties available in the three countries. Even though some degree of selection is practised by farmers, there is no strict isolation of such maize fields from the neighbouring farms so geneflow between maize plants within the same farm as well as between different farms is likely to occur. From this study, it can also be hypothesized that the landraces that grouped together with improved and introduced varieties (group 3) may actually be 'creolized' varieties, i.e. hybridizations between traditional landraces from groups 1 and 2 with improved varieties available in the three countries. From the three clusters identified from this study, the upper 25% of the landraces representing those with the highest average diversity of the clusters were used to form a subset for further field evaluation under drought, low soil fertility and acid soils.

Despite their importance, the genetic characterization of landraces has been largely ignored until very recently. Genetic characterization in maize landraces has been primarily characterized by using morphological traits (Lucchin et al. 2003, Franco et al. 1997). The results from the present study could also serve as reference data for future maize collections in Zimbabwe, Zambia and Malawi. An additional step to this study will be to characterize the level of diversity at the molecular level using DNA markers. Using for example SSR markers, the landraces containing unique alleles at a given loci will be identified and further characterized for markers associated with abiotic stress tolerance, as these are the populations most likely to contain new alleles in general and potentially for stress-related loci. The genetic characterization data will provide useful information for utilizing these populations in maize breeding programmes to create abiotic stress tolerant maize.

## References

- Aquino, P., F. Carrión, R. Calvo and D. Flores. 2001. Selected Maize Statistics. *In* CIMMYT Maize Facts and Trends. Mexico (D.F. Pingali, P.L., ed.). CIMMYT 1999–2000 World Maize Facts and Trends. Meeting World Maize Needs: Technological Opportunities and Priorities for the Public Sector. CIMMYT, Mexico, D.F.
- Beck, D., F.J. Betran, M. Banziger, M. Wilcox and G.O. Edmeades. 1997. From landraces to hybrids: Strategies for the use of source populations and lines in the development of drought tolerant cultivars. Pp. 369–382 *in* Developing drought tolerant and low N tolerant maize (G.O. Edmeades et al., eds.). CIMMYT/UNDP, Mexico D.F.
- Blum, A. and C.Y. Sullivan. 1986. The comparative drought resistance of landraces of sorghum and millet from dry and humid regions. *Annals of Botany* 57:853–846.
- Brown, A.H.D. and D.R. Marshall. 1995. A basic sampling strategy: theory and practice. Pp. 75–92 *in* Collecting Plant Genetic Diversity: Technical Guidelines (L. Guarino, V. Ramanantha Rao and R. Read, eds.). CAB International, Wallingford, UK.
- Crossa, J. 1989. Methodologies for estimating sample size required for genetic conservation of outbreeding crops. *Theoretical and Applied Genetics* 77:153–161.
- Franco, J.E., J. Crossa, J. Díaz, S. Taba, J. Villasen and S.A. Eberhart. 1997. A sequential clustering strategy for classifying gene bank accessions. *Crop Science* 37:1656–1662.
- Hartkamp, A.D., J.W. White, A. Rodríguez Aguilar, M. Bänziger, G. Srinivasan, G. Granados and J. Crossa. 2000. Maize Production Environments Revisited: A GIS-based Approach. CIMMYT, Mexico, D.F.

- Lucchin, M., G. Barcaccia and P. Parrini. 2003. Characterization of a flint maize (*Zea mays* L. convar. *mays*) Italian landrace: I. Morpho-phenological and agronomic traits. *Genetic Resources and Crop Evolution* 50:315–327.
- McCann, J. 2005. *Maize and Grace: Africa's encounter with a new crop, 1500-2000*. Harvard University Press. USA.
- Sanchez-Gonzalez, J.J., M.M. Goodman and J.O. Rawlings. 1993. Appropriate characters for racial classification in maize. *Economic Botany* 47:44–59.
- SAS Institute, Inc. 1996. SAS Proprietary Software Release 6.12. SAS Institute, Inc., Cary, NC.
- Weinmann, H. 1972. *Agricultural research and development in Southern Rhodesia, 1890-1923*. Department of Agriculture Occasional Paper 4. University of Zimbabwe, Harare.

## Sorghum diversity and adaptation to drought in West Africa

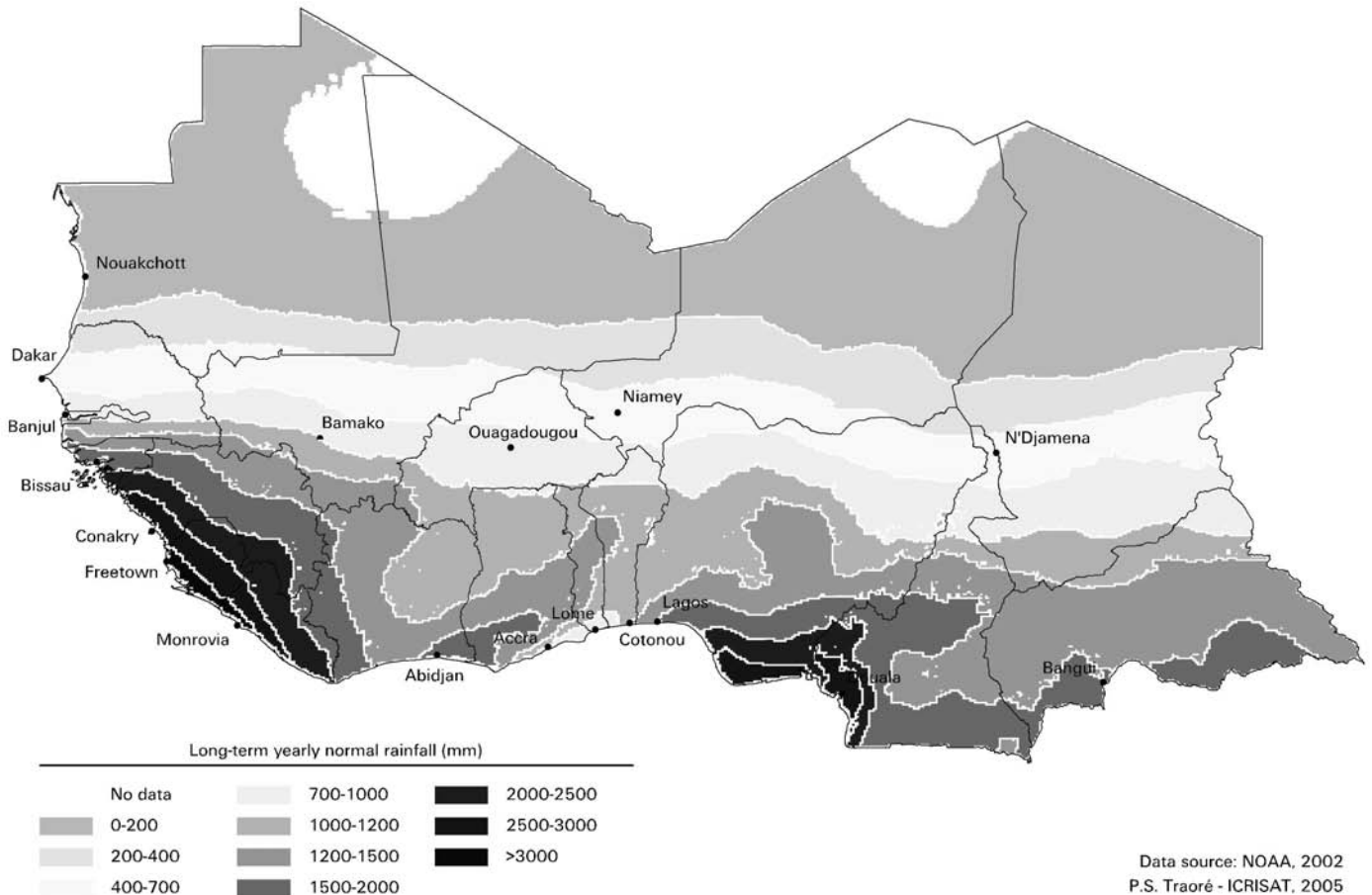
*E. Weltzien, H.F.W. Rattunde, B. Clerget, S. Siart, A. Toure and F. Sagnard*

### **Introduction**

Sorghum (*Sorghum bicolor*) and pearl millet (*Pennisetum glaucum*) are the main staple food crops of the West African Savannah zones. The West African Savannah is commonly subdivided into several agroecological zones, which are differentiated primarily by their total annual rainfall (Figure 1). The Sahelian zone (300–700 mm) is dominated by pearl millet cultivation, and has predominantly sandy soils. The Sudanian zone (700–1100 mm) is traditionally dominated by sorghum cultivation; however, in the southern Sudanian zone (>900 mm) maize cultivation is becoming more important in areas where access to fertilizers is facilitated. These zones extend all across West and Central Africa from west to east, in narrow bands practically parallel to the equator. In both zones the total annual rainfall varies considerably between years, but also the distribution within seasons is highly erratic. Specifically the onset of the rains in May/June is highly unpredictable. The end of the rainy season in contrast tends to be less variable, occurring within a 2–4 week period in all the zones. The zones also differ in the date of the end of the rainy season. In the drier Sahelian zone the normal date for the end of the rainy season arrives in the middle of September, whereas in the southern part of the Sudanian zone this may extend into the end of October.

Pearl millet and sorghum are the main staples of these zones. Pearl millet has its centre of origin in these zones, in the northern parts of the Sahelian zone, in areas where its wild progenitor is still found in abundance (Brunken et al. 1977). Pearl millet is cultivated on approx. 15 million ha each across West and Central Africa, providing the major source of calories, protein and minerals to the farming and herding populations in these zones across West Africa.

The Guinea-race of sorghum is predominantly cultivated in West Africa (de Wet and Huckabay 1967). This region is postulated to be the centre of origin of this race, where wild progenitors are common in the forest transition zones of the southern Sudanian zone (de Wet et al. 1972). The Guinea-race differs from the other four races of sorghum by its floret morphology at harvest: the maturing grains turn in the glumes by 45–90° so that the grain has minimal contact with the glumes. This trait facilitates threshing, but may cause grain shattering if the plants are left standing in the field too long after maturity. However, this trait is probably most relevant with respect to the drying of the grain, especially in the case of late rains, or unusually high humidity. Thus Guinea-race sorghums tend to be less prone to grain molds and discolouration of the pericarp from the coloured glumes surrounding the grains. The Guinea-race is the most widely distributed and most common race of sorghum in Africa.



**Figure 1. Annual average rainfall isohyets in West Africa.**

### ***Adaptation to the dominant patterns of drought in West Africa***

Guinea-race varieties of sorghum tend to be adapted to a range of stresses commonly found in West Africa: poor soil fertility, *Striga* infestation, low soil pH, lodging, and a range of diseases and pests, which tend to only have local importance. The key challenge for adaptation of Guinea-race sorghums and pearl millet of this region is to the unpredictability of the onset of the rainy season and the widely varying dates of sowing. For both of these crops it has been shown that West African varieties are capable of maintaining a fairly constant date of flowering despite widely differing sowing dates. This is possible due to the plants' response to daylength, and more specifically to the daily rate of change in the sunset hour (Clerget 2004), which determines the date of panicle initiation, and further reproductive development (Figures 2, 3). This sensitivity to the photoperiod does lead to the advantage for farmers that these varieties can be sown over a period of 3 months (mid-May to mid-August) and still flower within a 2-week period around the normal end of the rainy season (Figure 4).

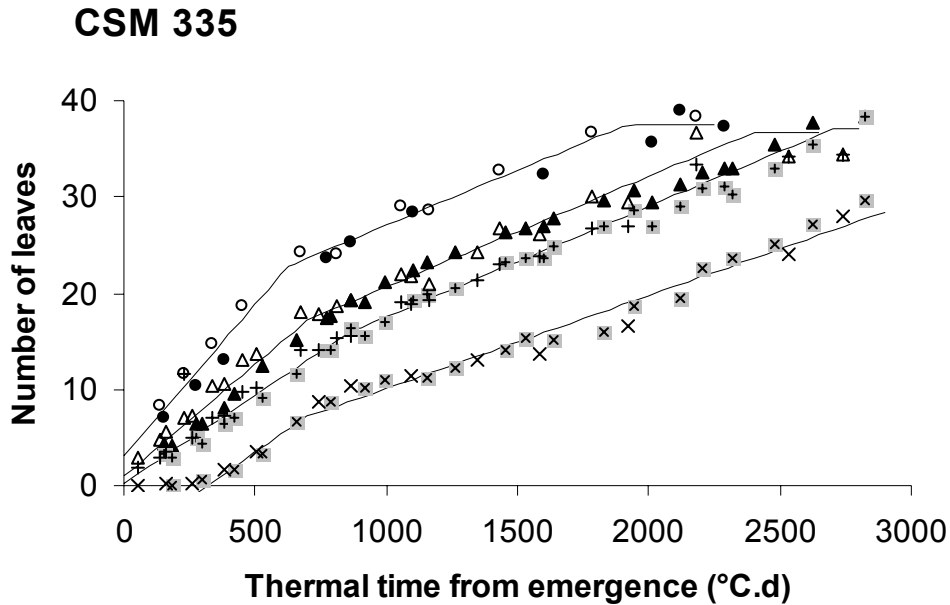


Figure 2. Relationships between the number of initiated ( $\circ$ ), appeared ( $\Delta$ ), ligulated ( $+$ ) and senesced ( $\times$ ) leaves on the main culm and the thermal time for the sorghum variety CSM 335 sown in March 2001 (open symbols) and March 2002 (closed symbols).

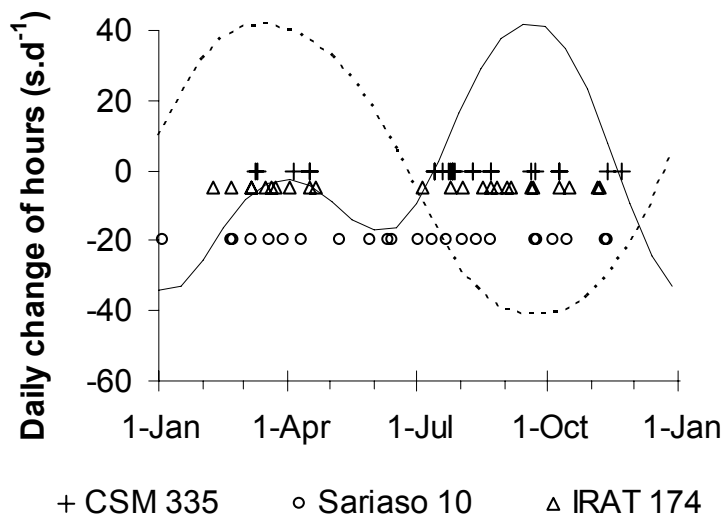


Figure 3. Relationships between the dates of occurrence of the panicle initiation observed from continuous monthly sowings and the daily change of the daylength (...) or the daily change of the sunset hour (—).

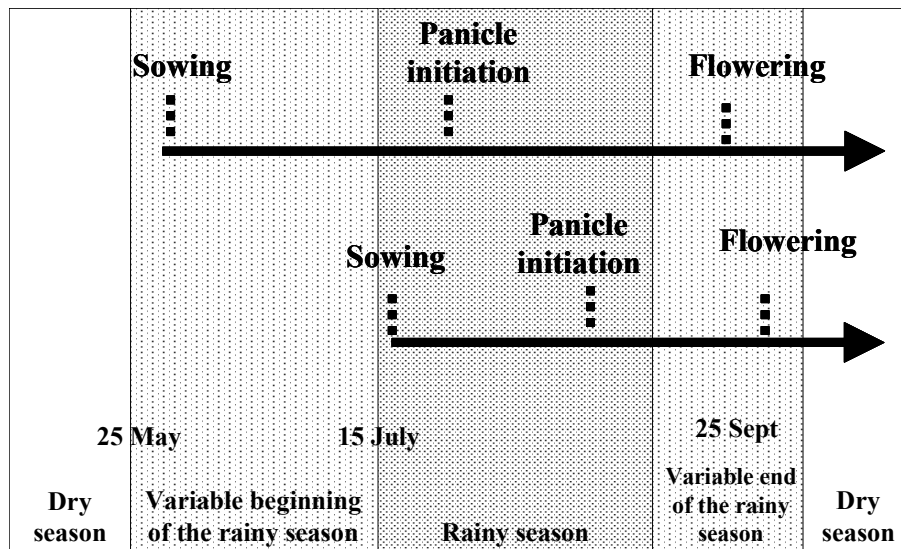


Figure 4. The local photoperiod-sensitive varieties flower nearly at the same date every year, independently from the sowing date, when the rainfall ends at the average time.

### ***Diversity patterns and drought***

During a recent survey, sorghum landraces were collected along a north-south gradient in Mali covering six villages from the Sahelian zone to the southern Sudanian zones. The survey showed that sorghum is being cultivated in all these agroecological zones. Guinea-race sorghums dominate the varietal portfolio of farmers, but other races of sorghum are present in all the zones (Table 1). An initial evaluation of the flowering date of these varieties, when grown in southern Mali (ICRISAT Samanko station near Bamako), indicates that all the sorghum varieties found in Sahelian zones are early flowering. However from the northern Sudanian zone onwards the data indicate that all the villages have two predominant groups of Guinea-race sorghum varieties: an early flowering group, which is less sensitive to photoperiodic differences, and a later flowering group, which is highly sensitive to changes in photoperiod.

Table 1. Sorghum race diversity in six villages across a north-south gradient in Mali as observed during a survey in 2004.

| Village name | Rainfall zone (mm/yr) | No. of varieties of: |              |             |
|--------------|-----------------------|----------------------|--------------|-------------|
|              |                       | Guinea-race          | bicolor race | other races |
| Pomorododiou | 600                   | 2                    | 1            | 4           |
| Kagnan       | 750                   | 11                   | 1            | 0           |
| Sougoumba    | 1000                  | 5                    | 1            | 0           |
| Dioulafoundo | 1100                  | 7                    | 2            | 0           |
| Siramana     | 1200                  | 7                    | 1            | 0           |
| Douasso      | 1300                  | 7                    | 1            | 0           |

### ***Adapting to climate changes: farmers' seed-management strategies***

A preliminary analysis of a study of farmers' seed systems in the Sudanian zone of southern Mali, based on interviews with 236 farmers in 13 villages in the Mande area, located between Bamako and the border to Guinea, provides insights into farmers' strategies to cope with variable climatic conditions.

The varietal diversity of sorghum at the local level is generally high in this area. About 40 different varieties were mentioned as being cultivated in the region at that time. These varieties had different names, and were described by farmers as having different



characteristics. Of the mentioned varieties 94% belong to the Guinea-race of sorghum. In each of the villages we found 5 to 12 different varieties. Individual families commonly cultivate one (40% of households), two (30%), or three varieties (22%). Families grow different varieties of sorghum to meet different needs such as:

- different maturity cycles, with the majority being full season varieties for 'normal' sowing dates, but an important minority of shorter cycle varieties for providing grain during the 'hungry period' before the main harvest, as well as for late sowing dates or changing growing conditions like the shortening of the rainy season
- adaptation to different types of soils, like bush fields, house fields, inland valleys, poor soils, *Striga*-infested soils, fertile soils, waterlogged fields, etc.
- specific uses, like animal feeding, sugar juice for children, construction, special food processing, etc.
- good storage traits for commercialization and stocks for poor years, etc.
- different culinary qualities, like content of flour, preparation of grains like rice.

Most farmers produce their own seed for sowing, and select panicles from their own fields before general harvest. Selected seeds are stored separately from the grain for consumption. Storing methods are efficient for conservation. Most farmers (91%) mentioned that they do not have important seed losses during storage.

The survey has shown that farmers change their sorghum varieties quite regularly. Their decision for changing varieties is usually based on trials which they conduct themselves. Of the farmers we interviewed, 96% reported that they are used to conducting these kinds of tests. Within a family, varieties are often kept for 10 years (49% of varieties), and sometimes longer.

More than 70% of farmers mentioned the date of maturity or growth duration as an important characteristic of their varieties. Only two varieties of the 40 actual cultivated varieties were described as being late maturing; all the others were characterized to have a short or intermediate cycle. 'Long cycle' or shortening of the rainy season was mentioned as the reason why 40% of the varieties were abandoned. Farmers explained their interest in 'fast', early maturing varieties with the observation that their old varieties with later maturity no longer correspond to the length of the current rainy season.

When tracking the origins of the different varieties cultivated, we found that the most common source for new varieties (40% of varieties) was other farmers in the same village, while 20% come from farmers in neighbouring villages. In this region, which is generally considered more traditional, only 25% of the varieties grown were reported as being a heritage in the family. Information sources about new varieties are for 68% of farmers the talks with other farmers, the fields of other farmers (64%), travels (46%), projects (38%) and extension services (31%).

These results indicate that climate change, in particular the shortening of the length of rainy season, has profound impacts on the portfolio of varieties grown by individual families, villages and in specific areas. Similar results by Khouressy et al. (2003) have shown that varietal diversity of sorghum is particularly under threat in the higher rainfall areas, where late-maturing, highly photoperiod sensitive varieties with 6 to 7-month cycles used to be commonly grown. It should be noted, however, that the sorghum varieties currently grown in this zone also are photoperiod sensitive, but flower at an earlier time.

### ***Increasing varietal diversity for drought environments through breeding***

Breeders have rarely attempted to utilize the diversity of the Guinea-race for improving productivity and yield stability of sorghum varieties for West African growing conditions. The ICRISAT and National Program sorghum breeders in West Africa are pursuing several approaches to achieve improvements in productivity and overall value to the farmer through novel guinea-race sorghum varieties.

**Participatory population improvement and variety development**

Population improvement is useful for simultaneous improvement of multiple traits and complexly inherited traits determined by many genes. Thus this is a promising approach to increasing yield of photoperiod-sensitive sorghums while maintaining selection pressure for adaptation and quality traits. Population improvement in sorghum has rarely been used, as sorghum is normally treated like other self-pollinated crops. However, recent studies with the Guinea-race sorghums have shown that outcrossing rates are significantly higher than in other races of sorghum (Ollitrault et al. 1997). Thus continued inbreeding is likely to result in some inbreeding depression, which is undesirable. We are thus exploring recurrent selection in broad base populations for varietal improvement, using genic male sterility to facilitate crossing and recombination.

A genetically broad-based Guinea-race sorghum population was bred by crossing 13 West African Guinea-race landrace varieties with a source of genic male sterility (MS3), followed by two generations of backcrossing to the landrace parents (Rattunde et al. 1997). Three cycles of recombination were conducted prior to initiating stronger mass selection, with male-sterile selections used to constitute the next cycle bulk, and male-fertile selections (S1 progenies) used for initiating variety development.

This guinea-race population has given rise to a series of new populations. Crosses of this population with farmers' varieties of later maturity and genebank accessions of landraces of diverse geographic origins and larger grain size produced a series of new populations. These introgressed populations provide source materials for more southerly (higher rainfall) zones and increase diversity for panicle and grain traits. These diversified population crosses are being used for farmer-managed mass selection in the population bulks, as an entry point for variety development. Variety development has also started on-station, by involving farmers in the evaluation of the progeny rows.

The original guinea-race population and the introgressed populations are all tall (plant heights of 3–5 m depending on sowing date), similar to traditional landrace varieties. A novel Dwarf Guinea Population was produced by selection of dwarf (shorter stem internode) plants segregating within the original tall population, intermating them and introgression of some bred varieties of dwarf height. This Dwarf Guinea Population is currently being improved through mass selection. Superior selections from the population are used for variety development, with replicated yield testing beginning at the S1 generation. The varieties now under test in multi-location farmer participatory tests show both high yield and superior stover quality for ruminant feeding. These new dual-purpose sorghums, providing both grain and improved stover, could further the crop-livestock integration that is starting in the region. Increased animal manure, and resulting improvements of soil tilth and water-holding capacity, could provide an indirect but significant contribution to reducing losses due to drought.

Now that these populations have achieved levels of adaptation and grain quality acceptable to farmers, we are initiating progeny-based recurrent selection for increased grain yields for the Sudanian zone of adaptation. Progenies will be derived from farmers' selections of selfed plants grown in their own fields. Evaluation of performance of these progenies is currently being conducted on research stations and in one farmers' field.

**Exploiting heterosis within the Guinea-race**

Although guinea-race sorghums possess a suite of adaptive traits that make them the predominant sorghums in West Africa, they give limited yield response to intensified production conditions. A promising approach to increase the productivity of Guinea-race sorghums while retaining the required adaptive and quality characteristics is the development of Guinea-race hybrids. Furthermore, previous research on sorghum hybrids with non-Guinea germplasm has shown that the yield advantage of sorghum hybrids can be

as great as or even greater under drought stress as under more optimal conditions (Hausmann et al. 1998).

Great strides have been made over the past five years to develop the first Guinea-race hybrids. The first series of male-sterile parents (A lines) has been developed, including 16 landraces and 7 inter-racial lines. The newly developed set of Guinea-race male-sterile A/B pairs were used to produce the first experimental hybrids, including Inter-Racial (Guinea × Caudatum-race materials), Inter-Racial × Guinea, and Guinea × Guinea hybrids. The best hybrids significantly outyielded all of the well-adapted check varieties in three on-station and one on-farm trial (including the predominant variety cultivated in that village). The fact that 2004 was a drought year further suggests the possibility that hybrids could provide useful contributions to achieving stable, high yields despite climatic variability.

### Increased harvest index of Guinea-race sorghums through the introduction of dwarfing genes

The traditional Guinea-race sorghum varieties are all tall, reaching heights of 4-5 m regularly, especially when sown early. Stover biomass of these varieties tends to be very high. Under fertile growing conditions, 10–15 t/ha of biomass are achieved, also in farmers' fields. However, harvest index of these varieties tends to be very low, often only 10–20%. The stover is used for construction, while the leaves are used as animal feed. However much of the stover remains in the field throughout the dry season, and is often burned just before the first rains arrive. This indicates that the value of the stover is actually very limited. We are thus exploring options to improve the value of the stover by increasing its quality as an animal feed. One promising option for achieving this goal is to introduce dwarfing genes into these sorghum populations. Recent research results have shown that significant improvements in stover quality are associated with a reduction in internode length, i.e. plant height. We expect that it shall also be possible to increase grain yield of these new dwarf guinea types using the recurrent population improvement methods as outlined above. In some villages farmers have expressed a strong interest in the new dwarf types (Figure 5).

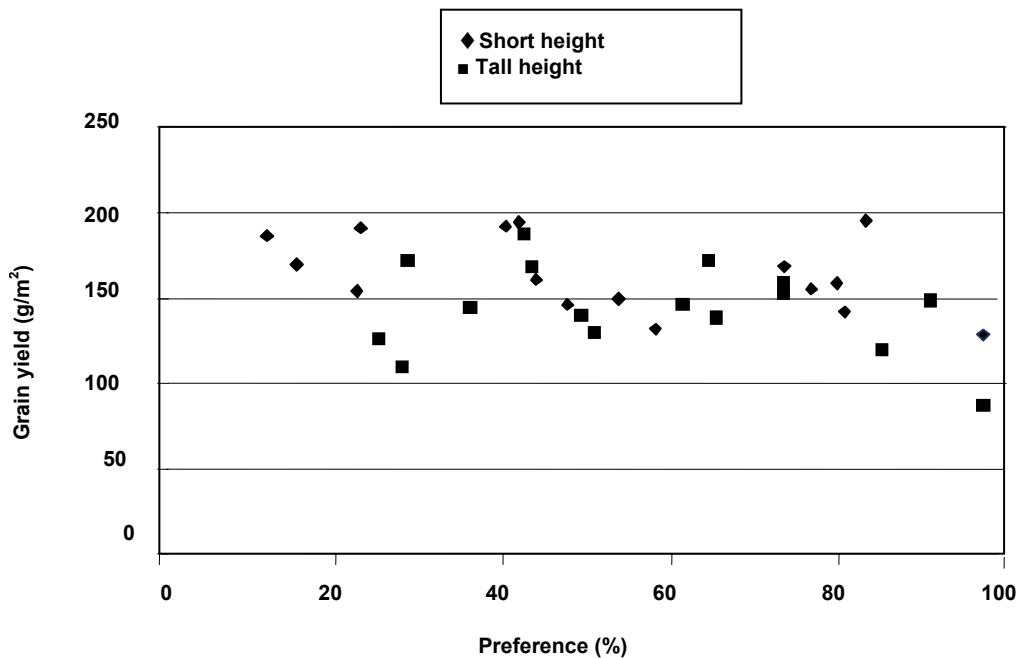


Figure 5. Farmers' preferences for varieties of tall and short height of Guinea-race sorghum with a range of grain yield performances, Kegnero 2004.

### **Creation and characterization of a Guinea-race core collection**

To facilitate the effective utilization of the genetic diversity within the Guinea-race of sorghum a Guinea core collection of 293 accessions was formed from the 3907 Guinea accessions in the ICRISAT Sorghum collection. The collection represents Guinea-race sorghums originating in West Africa, Eastern and Southern Africa as well as Asia. This core collection was evaluated for important agronomic characteristics in replicated trials by ICRISAT-Samanko, Mali, INERA-Farako Ba, Burkina Faso, INRAN-Bengou, Niger. Evaluations have confirmed the existence of great diversity for most traits of agronomic importance. Evaluations at ICRISAT-Mali, for example, showed flowering dates varying from September through November, plant height from 1.3 to 5.5m, panicle length from 20 to 61 cm, and 100-grain weight from 0.4 to 3.6 g. Limited variation was observed, however, for stem internode length (dwarfing).

### **Conclusions**

Drought patterns strongly shape the diversity and types of crops grown in any agricultural system. Sorghum and pearl millet in West Africa appear to have highly developed and very similar mechanisms of adaptation to the particular rainfall distribution in the Sahelian and Sudanian Savannah zones of West Africa. Their capacity to modify the length of their vegetative phase to achieve a nearly constant calendar date of flowering despite very contrasting sowing dates, assures that grain maturity corresponds with the end of the rainy season. This is one of the most important adaptive characteristics. Furthermore, sorghum producers minimize risks due to climatic variability by using a considerable range of varieties with different flowering dates. They also use several crops that differ for patterns of water needs and responses to water stress. Assessing the usefulness of diversity for drought situations requires a thorough understanding of the drought patterns over years and farmers' options for effectively using the available water for food production. Understanding farmers' strategies for varietal choice and seed production also provides insights into the importance of inter- and intravarietal diversity in drought-prone areas.

### **References**

- Brunken, J.N., J.M.J. de Wet and J.R. Harlan. 1977. The morphology and domestication of pearl millet. *Economic Botany* 31:163–174.
- Clerget, B. 2004. Le rôle du photopériodisme dans l'élaboration du rendement de trois variétés de sorgho cultivées en Afrique de l'Ouest. Ecole Doctorale ABIES, Paris, France. [<http://pastel.paristech.org/view/institution/inapg.html>], 103 pp.
- de Wet, J.M.J, J.R. Harlan and B. Kurmar Dohita. 1972. Origin and evolution of Guinea race sorghums. *East African Agricultural and Forestry Journal* October: 114–119.
- de Wet, J.M.J. and J.P. Huckabay. 1967. The origin of *Sorghum bicolor*. II. Distribution and domestication. *Evolution* 21(4):787–802.
- Hausmann, B.I.G., A.B. Obilana, A. Blum, P.O. Ayiecho, W. Schipprack and H.H. Geiger. 1998. Hybrid performance of sorghum and its relationship to morphological and physiological traits under variable drought stress in Kenya. *Plant Breeding* 117:223–229.
- Kouressy, M., D. Bazile, M. Vaksman, M. Soumaré, T. Doucouré et A. Sidibé. 2003. La dynamique des agrosystèmes : un facteur explicatif de l'érosion variétale du sorgho. P. 14 in *Organisation spatiale et gestion des ressources et des territoires ruraux*. CD-ROM, CIRAD, Montpellier, France.
- Ollitrault, P., J.L. Noyer., J. Chantereau and J.C. Glaszmann. 1997. Structure génétique et dynamique des variétés traditionnelles de sorgho au Burkina Faso. Pp. 231–240 in *Actes du colloques gestion des ressources génétiques des plantes en Afrique*.
- Rattunde, H.F.W., E. Weltzien R., P.J. Bramel-Cox, K. Kofoid, C.T. Hash, W. Schipprack, J.W. Stenhouse and T. Presterl. 1997. Population improvement of pearl millet and sorghum: current research, impact and issues for implementation. Pp. 188-212 in *Proceedings of the International Conference on Genetic Improvement of Sorghum and Pearl Millet*.

## Environmental effect and genotypic variation of maize on-farm in Peruvian Central Amazon

Jose Luis Chavez-Servia, Luis A. Collado-Panduro, Roger Pinedo-Ramirez, Ricardo Sevilla-Panizo, Wilfredo Guillén-Huachua and Jaime Mori-Castro

### Introduction

The *riverero* peasants, who live in small communities along the rivers in the Central Peruvian Amazon, depend completely on cultivated resources. Among these, maize plays a significant two-fold role in household livelihood, for home consumption and as a source of income. The *riverero* community is composed of the Shipibo, Ashaninka and Cashibo-Cactaibo ethnic groups who share territory with migrants from the Andean region, locally known as *mestizo* or *colonos*. Such ethnic diversity is also reflected in maize diversity. For instance, native tropical maize native landraces and old and modern improved varieties introduced from the Andes and Brazil (Collado-Panduro et al. 2004; Chavez et al. 2004) have formed a genepool to confront one of the main environmental stress forces in the region—drought during mid and (sometimes) terminal growth period of maize. Drought, like many other environmental stress conditions, has adversely affected lowland maize yields. This can be appreciated through visual indicators of water deficit in maize such as acceleration of the senescence of lower leaves, leaf rolling, delay in silking days, increase of anthesis–silking interval, poor grain filling, shorter ears, and lower kernel number per ear, kernel weight, ears per plant and plant height (Bolaños et al. 1993; Bolaños and Edmeades 1993, 1996; Chapman et al. 1997; Sari-Gorla et al. 1999). Moreover, the work of CIMMYT during the 1990s on varietal improvement of maize suggests that combination of attributes such as anthesis–silking (ASI) and ears per plants positively correlated with grain yield can confer mid- and late-season drought tolerance (Chapman et al. 1997).

At least three genepools have been identified in the central Peruvian Amazon: (1) Piricincó and (2) Cuban Yellow Dent races were reported by Grobman et al. (1961) and their presence was recently confirmed in Ucayali, Huanuco and Pasco Departments (Chavez et al. 2004). Further, Collado-Panduro et al. (2004) and Chavez et al. (2004) provided evidence of a third pool known as *Canchas* but with morphological characteristics similar to a Brazilian and Uruguayan indigenous race called Pipoca, from the group *Avati Pinchinga Ihu* (Brieger et al. 1958; Paterniani and Goodman 1977).

Evaluations on-farm served as a methodological base for the work presented here, which demonstrates that variation in rainfall in the Peruvian Central Amazon has an influence on maize performance. Agroecosystems of *rivereros* peasants have a maize cultivation period of 120 days (mid-May to early September) and depending on land elevation (light highland or lowland) there is an effect of river floods and water stress. Environment is thus the determinant of maize yield and the genotypes can respond in different ways to the variation of rainfall, soil characteristics and management practices which define the different types of genotype × environment interactions (Lacaze and Roumet 2004). This paper summarizes the evaluation of the environmental effect of two precipitation regimes on the genotypic variation of 68 samples of landraces and two improved varieties (controls) of maize in Ucayali, Peru.

## Materials and methods

### Plant material

Evaluated material (maize sample populations) was donated by local farmers in three different village areas of Peruvian Central Amazon, the Ucayali and Aguaytia Valleys within the Department of Ucayali Province, and the Pichis-Pachitea valley between the Departments of Pasco and Huanuco. Each sample was considered as a different population, represented by an average of 30 ears, making a total of 68 populations (Table 1) plus two checks denominated as 'Pipoca' and 'Marginal Tropical 28', which do not appear in Table 1.

**Table 1. Geographic location of the origin of 68 maize landraces samples evaluated in Ucayali, Peru, 2003.**

| Subregions             | Geographic area of origin of the samples (longitude and latitude) †   | Preliminary racial classification of samples |           |                     | Total of samples |
|------------------------|---|--|-----------|---------------------|------------------|
|                        |   | Cubano Amarillo                              | Piricinco | Avati Pichinga Ihu‡ |                  |
| Ucayali Valley         | From Colonia de Caco (74.30 and 9.34) to Nuevo Ceylan (74.27 and 8.62) along Ucayali River                  | 14   | 13        | 6                   | 33               |
| Aguaytia Valley        | From San Jose de Tunuya (74.94 and 8.38) to Patria Nueva, Calleria (74.58 and 8.0) following Aguaytia River | 11   | 4         | 2                   | 17               |
| Pichis-Pachitea Valley | From Puerto Ocopa (74.98 and 10.06), Pasco to Cleyton (74.94 and 9.2), Huanuco                              | 14   | 3         | 1                   | 18               |

† Longitude and latitude in degrees and minutes, west and south, respectively.

‡ Also known as Pipoca.

### Evaluation of environments and recorded data

Evaluated agroecosystems are locally known as *restingas* (natural levee) of *Altura* (high) and *Baja* (low), located in the region surrounding the villages of Nueva Requena and Nuevo Ahuaypa in the Department of Ucayali. The predominating climate in both is tropical humid (MINAG-INRENA 1995). Experiments were conducted in 2003 under two contrasting rainfall conditions: Nueva Requena region had 27 continuous days without rainfall (drought) and Nuevo Ahuaypa area had the optimal rainfall (well-watered) for maize. For environmental conditions other than rainfall, pH, texture, and soil nutrients, both regions present similar conditions (Table 2). The crop growing period was from June to October 2003 for Nueva Requena and from June to November of the same year for Nuevo Ahuaypa.

Days to anthesis (DA), days to silking (DS), anthesis-silking interval (ASI), plant height (PH), ear height (EP), ears per plant (EPP), 100-kernel weight (KP), ear length (EL), ear diameter (ED), kernel length, width and thickness (KL, KW and KT), and yield (Y) were evaluated in both experiments. ASI was computed as the difference in days between DA and DS.

### Farmers' observations in the drought environment

Maize samples in the drought experimental station in Nueva Requena were empirically evaluated by 20 local farmers (14 men; 6 women). Through mainly a visual assessment, each of the male and female farmers individually expressed which populations they would select as they believed these reach better agronomic performance (outstanding varieties), even though plants were growing in conditions of water stress. Evaluated samples were reaching last phase of silking and grain filling. From the total of outstanding populations selected by the farmers and the number of times each population was selected, a 'preference percentage'

was calculated. The experimental plots were not labelled with variety names and every farmer had the freedom to select the plots he/she wanted. In the well-watered environment there were not favourable social conditions to make the same evaluation.

**Table 2. Description of the evaluation environments in Ucayali, Peru, 2003.**

| Site characteristics   | Nueva Requena                 | Nuevo Ahuaypa                 |
|--|-------------------------------|-------------------------------|
| Latitude south   | 8°18'39"                      | 9°4'45"                       |
| Longitude west   | 74°51'05"                     | 74°28'02"                     |
| Altitude (masl)  | 160                           | 175                           |
| Average temperature (°C)   | 27                            | 32                            |
| Cumulative precipitation (mm) †  | 307                           | 815                           |
| Days continue without precipitation (mid-season drought)                               | 27                            | 0                             |
| Physical texture of soils  | Silty clays                   | Sandy loams                   |
| Availability of macro-elements in the soil   | Low                           | High                          |
| pH   | 5.4                           | 6.5                           |
| Type of agroecosystem <i>restinga</i> (natural levee referred to the floodplain level) | Altura (above the floodplain) | Baja (on or under floodplain) |
| Definition of environment  | Drought                       | Well-watered                  |

† Precipitation recorded during June–October in Nueva Requena and June–November in Nuevo Ahuaypa 2003. Source: Laboratory of soil and plant analysis of INIEA-Pucallpa.

### Data analysis

Evaluated maize samples in Nueva Requena and Nuevo Ahuaypa were distributed on-farm following a design of rectangular simple lattice with two replications. Trials were analyzed under a lineal mixed model of randomized complete blocks because the lattice design did not exceed 5% of the efficiency of the randomized blocks (SAS 2000). In order to carry out a combined analysis of variance (ANOVA), evaluated populations were considered as fix effects, evaluation environments as a random effect and repetitions nested in environments (Sahagun 1998), using the PROC GLM (SAS 2000).

From the variance components of ANOVA and using the moments method (Sahagun 1998), were estimated the genotypic variation from evaluated populations ( $\sigma_G^2$ ), environmental variance ( $\sigma_E^2$ ) and variance of the genotype  $\times$  environment interaction ( $\sigma_{G \times E}^2$ ). From these were calculated the broad-sense heritability ( $H^2$ ), repeatability ( $r$ , according to Goodman and Paterniani 1969) and the coefficient of genotypic variation (CV) through these expressions:  $\sigma_G^2 = [MS_G - MS_{ExG}] / r \times \underline{e}$ ;  $r$ , replications and  $\underline{e}$  environments;  $\sigma_E^2 = [MS_E - MS_r] / r$ ;  $\sigma_{G \times E}^2 = [MS_{ExG} - MS_{error}] / r$ ;  $H^2 = [\sigma_G^2] / [\sigma_G^2 + \sigma_E^2 + \sigma_{G \times E}^2]$ ;  $r = [\sigma_G^2] / [\sigma_E^2 + \sigma_{G \times E}^2]$ ; and  $CV (\%) = \sqrt{[\sigma_G^2] / \bar{X}} * 100$  ( $\bar{X}$ , mean).

Evaluated samples were classified based on morphological variability through a cluster analysis as suggested by Franco et al. (1997) and Sanchez et al. (2000). From the agromorphological variables (excluding yield) and the corresponding Euclidian distances, a dendrogram was generated by the average link method. The analysis was performed using standardized variables.

### Results

The ANOVA showed statistically significant differences ( $p=0.01$ ) among the evaluated populations as well as between the evaluated environments for all variables (statistics not shown). That is, evaluated samples presented a differential response while the environments were significantly different.

### Genotypic and environmental variation

Evaluated environments were determinants for phenotypic expression. Environmental variance had at least 85% of the total phenotypic variance in each of the evaluated characteristics (Table 3). This suggests that the evaluated germplasm was exposed to contrasting yet restrictive water conditions, e.g. Nueva Requena was 27 days without rainfall, affecting plant development and yield. It is important to mention that even under such conditions, repeatability ( $r$ ) of the anthesis–silking interval characteristics and ears per plant was constant. In fact, even under limitations of precipitation the ASI and EPP are traits to consider in the selection of stable genotypes.

Broad-sense heritability ( $H^2$ ) showed a variation 0.002 to 0.65. The highest generated value was for the EPP. Also, in the  $H^2$  calculation, there is a notorious influence of the environmental variance compared with the genetic variance and the genotype  $\times$  environment interaction (Table 3).

**Table 3. Estimators of the genetic and environmental variation in the maize samples evaluated in Ucayali, Peru, 2003.**

| Variable                  | $\sigma_G^2$ | $\sigma_E^2$ | $\sigma_{G \times E}^2$ | $H^2$ | $r$   | CV   |
|---------------------------|--------------|--------------|-------------------------|-------|-------|------|
| Anthesis                  | 3.23         | 1555.0       | 1.49                    | 0.002 | 0.002 | 2.9  |
| Silking                   | 3.83         | 2133.4       | 2.17                    | 0.002 | 0.002 | 3.0  |
| Anthesis–silking interval | 0.068        | 38.7         | 0.296                   | 0.002 | 0.002 | 7.7  |
| Plant height              | 585.0        | 163972.4     | 108.4                   | 0.004 | 0.004 | 9.9  |
| Ear height                | 292.6        | 101713.7     | 61.3                    | 0.003 | 0.003 | 12.4 |
| Ears per plant            | 0.052        | 0.02         | 0.01                    | 0.647 | 1.831 | 27.5 |
| 100-kernel weight         | 30.38        | 2366.42      | 5.20                    | 0.013 | 0.013 | 21.5 |
| Ear length                | 12.50        | 383.17       | -0.042                  | 0.032 | 0.033 | 21.3 |
| Ear diameter              | 0.462        | 12.113       | -0.196                  | 0.037 | 0.039 | 18.9 |
| Kernel length             | 0.012        | 1.467        | 0.003                   | 0.008 | 0.009 | 11.4 |
| Kernel width              | 0.011        | 0.340        | <0.001                  | 0.032 | 0.034 | 13.0 |
| Kernel thickness          | 0.007        | 0.098        | <0.001                  | 0.066 | 0.070 | 17.3 |
| Yield                     | 122818       | 69107634     | 118897                  | 0.002 | 0.002 | 20.7 |

$\sigma_G^2$ ,  $\sigma_E^2$  y  $\sigma_{G \times E}^2$ , means genotypic, environmental and interaction genotype–environment variances, respectively;  $H^2$ , heritability;  $r$ , repeatability; and CV, coefficient of genetic variation.

For the drought environment (Nueva Requena), an average of 965 kg/ha was estimated for grain yield, very low compared with 2446 kg/ha of the non-drought environment (Nuevo Ahuaypa), giving a difference in yield of about 60%. Such a difference gives an estimate on how drought can directly affect yield. However, drought might not be the only force affecting yield. Other estimators of the degree of effect are delay in days to silking and anthesis, increase of the ASI, lower longitude and diameter of ear and lower ear and plant height (Table 4).

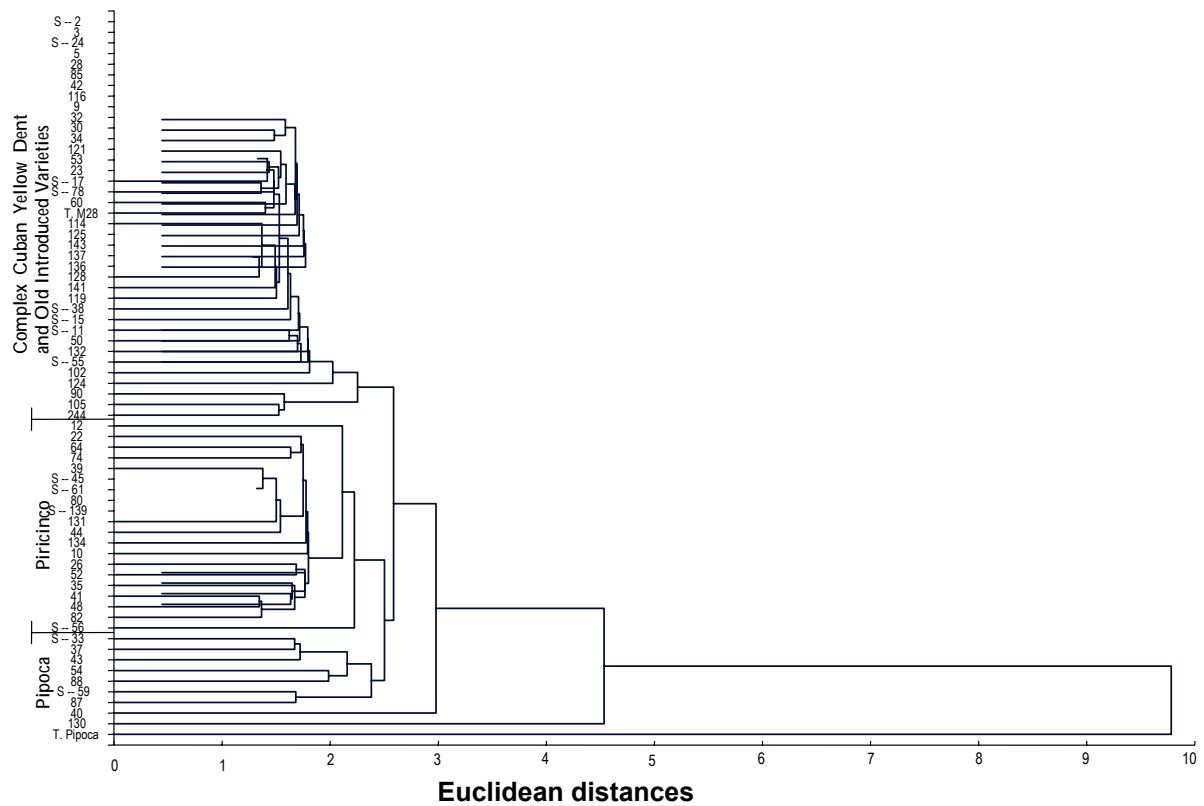
### Clustering by morphological variation

The cluster analysis carried out confirms the *a priori* classification in Table 1. A significant morphological difference was identified among the groups named ‘Pipoca’, ‘Piricinco’ and the complex comprised of ‘Cuban Yellow Dent’ and introduced varieties. Regarding phenotype, there were no ears showing grain combinations of the above-mentioned varieties (Figure 1). Group names have been given according to the most frequent name within the group.



**Table 4. Means of the evaluated variables in two environments.**

| Variables                        | Drought environment<br>(Nueva Requena) | Well-watered environment<br>(Nuevo Ahuaypa) | Average |
|----------------------------------|--|---|---------|
| Anthesis (days)                  | 64.40                                  | 57.57                                       | 61.03   |
| Silking (days)                   | 68.34                                  | 60.45                                       | 64.45   |
| Anthesis–silking interval (days) | 3.94                                   | 2.87  | 3.42    |
| Plant height (cm)                | 209.80                                 | 279.60                                      | 244.20  |
| Ear height (cm)                  | 111.10                                 | 166.39                                      | 138.34  |
| Ears per plant                   | 0.82                                   | 0.85  | 0.83    |
| 100-kernel weight (g)            | 21.52                                  | 29.93                                       | 25.67   |
| Ear length (cm)                  | 14.97                                  | 18.30                                       | 16.61   |
| Ear diameter (cm)                | 3.29                                   | 3.91  | 3.60    |
| Kernel length (mm)               | 0.88                                   | 1.09  | 0.98    |
| Kernel width (mm)                | 0.77                                   | 0.87  | 0.82    |
| Kernel thickness (mm)            | 0.50                                   | 0.45  | 0.48    |
| Yield (kg/ha)                    | 964.83                                 | 2446.39                                     | 1689.39 |



**Figure 1. Dendrogram of 68 samples of maize landraces and two controls, based on 13 morphological traits and in the average link method of Euclidean distances. S, simples with higher yielding and T= checks (Pipoca y Marginal 28 –M28-).**

The 'Piricinco' maize race has been diversified in the Ucayali region, where it is locally known as *Suave*. This race showed a yield statistically similar to that of the check (Marginal 28), giving evidence of the potential adaptability of local varieties of the race 'Piricinco' to the micro-niches where it is continuously evolving. Table 5 lists the more stable material regarding yield. Check Marginal 28 was shown to be statistically similar to one group within the complex referred to as Cuban Yellow Dent (CZ078CA, CZ002EA, CZ011CA and

CZ024EA). From the Piricincó, CZ061LC, CZ056LC and CZ139CP were outstanding and from the group Pipoca, CZ059LC. Within the evaluated group, there was material identified as earlier than the checks. Further, the evaluation makes evident that there exists wide genotypic variability on-farm. In general, there were more differences among than within the morphological groups.

**Table 5. Maize populations averaging highest yields over environments, Ucayali, Peru, 2003.**

| Cluster †                               | Sample<br>code | Drought (Nueva Requena) |      |        | Well-watered (Nuevo<br>Ahuaypa) |      |        | Avg.<br>yield |
|---|----------------|-------------------------|------|--------|---------------------------------|------|--------|---------------|
|   |                | DA ‡                    | EL ‡ | Y ‡    | DA                              | EL   | Y      |               |
| Complex                                 | CZ078CA        | 63.0                    | 13.3 | 1386.3 | 57.0                            | 17.9 | 3673.1 | 2529.7        |
| Cuban Yellow                            | CZ002EA        | 63.5                    | 13.6 | 836.9  | 58.5                            | 17.6 | 4059.4 | 2448.1        |
| Dent and old<br>introduced<br>varieties | CZ011CA        | 62.0                    | 14.4 | 1595.9 | 56.5                            | 17.1 | 3227.2 | 2411.6        |
|   | CZ024EA        | 64.5                    | 13.6 | 905.3  | 56.0                            | 17.2 | 3892.8 | 2399.0        |
|   | CZ017CA        | 63.0                    | 15.1 | 1151.3 | 56.5                            | 18.1 | 3412.2 | 2281.8        |
|   | CZ015LC        | 62.5                    | 14.4 | 1143.5 | 56.0                            | 17.8 | 3219.2 | 2181.4        |
|   | CZ038CA        | 67.0                    | 14.8 | 1016.7 | 56.5                            | 17.0 | 3197.3 | 2107.0        |
|   | CZ055LC        | 64.0                    | 14.0 | 658.3  | 56.5                            | 17.1 | 3213.7 | 1936.0        |
| Piricincó                               | CZ061LC        | 61.5                    | 17.5 | 1659.1 | 55.5                            | 21.5 | 2536.4 | 2097.8        |
|   | CZ056LC        | 63.5                    | 20.8 | 1439.0 | 59.0                            | 24.4 | 2503.2 | 1971.1        |
|   | CZ139CP        | 61.0                    | 19.9 | 1389.3 | 57.5                            | 22.7 | 2453.5 | 1921.4        |
|   | CZ045CA        | 63.5                    | 19.5 | 1078.3 | 58.0                            | 23.1 | 2433.5 | 1755.9        |
| Pipoca o Avati                          | CZ059LC        | 69.5                    | 13.8 | 1012.4 | 61.0                            | 15.7 | 3333.2 | 2172.8        |
| Pichinga Ihu                            | CZ033CA        | 65.0                    | 16.0 | 587.2  | 59.0                            | 18.4 | 2890.0 | 1738.6        |
| Checks                                  | Marginal 28    | 60.5                    | 14.1 | 1236.1 | 55.0                            | 17.7 | 4212.6 | 2724.4        |
|   | Pipoca         | 54.0                    | 10.3 | 137.4  | 54.5                            | 13.9 | 493.4  | 315.4         |

† Group identified in the cluster analysis.

‡ DA, days to anthesis; EL, ear length; and Y, yield (kg/ha).

### Farmers' observations in the drought environment

While male and female farmers were walking around the experiment of Nueva Requena (drought environment), they freely chose those populations they considered outstanding. According to their comments, the most visible indicators for them were plant size (height), stem diameter and ear length and width, among others. All farmers together selected a total of 31 populations as the researcher asked them to select more than one option. Every farmer selected an average of five populations. The populations or samples receiving more attention were: *Mejorado* (improved)—more than 5 years under cultivation—and *Maiz de Peso* (weight maize) which belongs to the Cuban Yellow Dent race; two *Suaves* (soft) within the Piricincó race and one called *Cancha Amarillo* (yellow toasted maize). Checks were planted but farmers never select them (Table 6).

### Discussion

In the Peruvian Amazon regularly more than 1000 mm of precipitation is recorded annually, which indicates that there never is drought affecting maize yield. However, the rainfall distribution is more important, as 27 consecutive days without precipitation results in a decrease in maize production. The same finding was reported by Narro et al. (1996) for maize in the region of San Martín, Cajamarca and Amazonas, Peru. According to these findings, rainfall distribution has a direct effect on maize production in the Peruvian Amazonian because this region is not solely jungle.

The Nueva Requena environment with a drought period lasting 27 days was a determining factor in the agromorphological performance of the evaluated maize samples. The environmental variance was significantly higher than the genetic variance and the genotype × environment interaction. Moreover, generated values of the above also had an influence over the broad-sense heritability estimate. Drought environment effect was higher

for the variables number of days to anthesis and silking, increase of anthesis–silking interval, lower plant and ear height and lower ear length and diameter. The most stable character was ears per plant.

**Table 6. Maize populations selected as 'potential' by farmers (N=20) under drought conditions, Nueva Requena, 2003.**

| Simple code | Local name    | % of preference | Simple code | Local name      | % of preference |
|-------------|---------------|-----------------|-------------|-----------------|-----------------|
| CZ031CA     | Mejorado      | 10.9            | CZ044CA     | Amarillo Suave  | 1.6             |
| IZ244SF     | Maíz de Peso  | 10.3            | CZ134CP     | Chuncho         | 1.6             |
| CZ039CA     | Suave         | 10.0            | CZ052LC     | Amarillo Suave  | 1.4             |
|             | Cancha        |                 |             | Shinki Kiteriri |                 |
| CZ088LC     | Amarillo      | 9.5             | CZ139CP     |                 | 1.4             |
| CZ061LC †   | Suave         | 6.3             | CZ015LC     | Estaquilla      | 0.7             |
| CZ050CA     | Piedra        | 5.7             | CZ131CP     | Amarillo        | 0.7             |
| CZ100PA     | Suave         | 5.6             | CZ132CP     | Duro            | 0.7             |
| CZ114CP     | Híbrido       | 5.1             | CZ013CA     | Híbrido         | 0.5             |
| CZ011CA     | Híbrido       | 5.1             | CZ019LC     | Suave           | 0.5             |
| CZ064CA     | Suave         | 4.4             | CZ029LC     | Serrano         | 0.5             |
| CZ045CA     | Suave         | 3.5             | CZ037CCH    | Cancha          | 0.5             |
| CZ023EA     | Serrano       | 2.8             | CZ038CA     | Serrano         | 0.5             |
| CZ017CA     | Amarillo      | 2.4             | CZ040CA     | Cancha          | 0.5             |
| CZ022EA     | Suave         | 2.4             | CZ059LC     | Cancha Blanco   | 0.5             |
| CZ054LC     | Cancha Blanco | 2.3             | CZ128CP     | Duro            | 0.5             |
| CZ009EA     | Híbrido       | 2.3             |             |                 |                 |

† Populations marked with bold font correspond to the same material selected in Table 5.

Morphological variability of the evaluated samples was classified into three main groups: (1) complex Cuban Yellow Dent and introduced varieties, (2) Piricinco race, and (3) Pipoca or *Avati pinchinga ihu*. Group variations were found across the environments. The maize known as *Canchas* or *Pipocas* was probably brought from Brazil. There exists an exchange area between Department of Ucayali, Peru and the State of Acre in Brazil through Rio Branco and Cruzeiro do Zul. Pipoca material evaluated here is similar in morphology to the material described by Paterniani and Goodman (1977) and Brieger et al. (1958), and yet these authors place Pipoca within the indigenous landraces *Avati Pichinga Ihu*. Samples from the Piricinco group belong to the race of the same name described by Grobman et al. (1961). The complex named Cuban Yellow Dent and introduced varieties of the Andean region integrated a group which combines materials with characteristics of the Cuban Yellow Dent and combinations of grain forms of the Andean maize. Grobman et al. (1961) reported that Cuban Yellow Dent was introduced in 1942 in the area of Tingo Maria (near the collection sites of the samples evaluated), and from there, was rapidly spread over the Central Amazon.

Farmers used vegetative and ear traits to choose five maize samples including two *Suaves* materials and one *Cancha*—both types used for home consumption, and two materials belonging to Cuban Yellow Dent, which are sold in the local market. In addition, seven of the selected samples were, through statistical procedures, also selected visually by farmers in Nueva Requena. The opinions, observations and selections made by the male and female farmers in Nueva Requena (drought environment) where specific materials were chosen as outstanding populations suggest that the researchers must take into consideration farmers' knowledge and experience. Both together are capable of solving and proposing potential solutions to face drought constraints in the region under question.

## Conclusions

The results presented here demonstrate that rainfall variations in the Central Peruvian Amazon do have an influence in maize morphology and agronomic performance. The effects of 27 consecutive days without rain in Nueva Requena were lower average yield, more days to anthesis and silking, increase of the anthesis–silking interval, lower plant and ear height, and lower ear length and diameter. Number of ears per plant was the most stable character according to the repeatability index ( $r$ ) and broad-sense heritability ( $H^2$ ). Genotypic and genotype  $\times$  environment variances were significantly lower than environmental variance due especially to the influence of the drought environment. In contrast, Nuevo Ahuaypa presented a favourable environment and soil characteristics for maize production. Despite the environmental influence, the maize samples were very stable (see Table 5). Morphological variability of the evaluated maize samples was clustered in three groups: (1) complex Cuban Yellow Dent and introduced varieties, (2) *Suaves* (Piricinco race), and (3) *Canchas*, *Pipoca* or *Avati Pinchinga Ihu* indigenous race.

## References

- Bolaños, J. and G.O. Edmeades. 1993. Eight cycles of selection for drought tolerance in lowland tropical maize. II. Responses in reproductive behavior. *Field Crop Research* 31:253–268.
- Bolaños, J. and G.O. Edmeades. 1996. The importance of the anthesis–silking interval in breeding for drought tolerance in tropical maize. *Field Crop Research* 48:65–80.
- Bolaños, J., G.O. Edmeades and L. Martinez. 1993. Eight cycles of selection for drought tolerance in lowland tropical maize. III. Responses in drought-adaptive physiological and morphological traits. *Field Crop Research* 31:269–286.
- Brieger, F.G., J.T.A. Gurgel, E. Paterniani, A. Blumenshein and M.R. Alleoni. 1958. Races of maize in Brazil and other eastern South American countries. Publication 593, National Academy of Sciences, National Research Council. Washington, DC, USA.
- Chapman, S.C., J. Crossa, K.E. Basford and P.M. Kroonenberg. 1997. Genotype by environment effects and selection for drought tolerances in tropical maize. II. Three-mode pattern analysis. *Euphytica* 95:11–20.
- Chavez-Servia, J.L., L. Collado-Panduro and R. Pinedo-Ramirez. 2004. Conservacion o perdida del valor de las variedades locales de los cultivos amazonicos. Pp. 503–537 *in* Peru: Problema Agrario en Debate – SEPIA X (F. Eugen, M.I. Remy and P. Oliart, eds). Seminario permanente de Investigacion Agraria (SEPIA). Lima, Peru.
- Collado-Panduro, L.A., R. Pinedo-Ramirez, J.L. Chavez-Servia and R. Sevilla. 2004. Diversidad genética de maíz en el Amazonas central peruano. P. 26 *in* Resúmenes de la XX Reunion Latinoamericana de Maíz 11–14 de October de 2004. Instituto Nacional de Investigacion y Extension Agraria, Universidad Nacional Agraria La Molina. CONCYTEC and CIMMYT, Lima, Peru.
- Franco, J., J. Crossa, J. Diaz, S. Taba, J. Villaseñor and S.A. Eberhart. 1997. A sequential clustering strategy for classifying gene bank accessions. *Crop Science* 37:1656–1662.
- Goodman, M.M. and E. Paterniani. 1969. The races of maize. III. Choice of appropriate characters for racial classification. *Economic Botany* 23:265–273.
- Grobman, A., W. Salhuana and R. Sevilla in collaboration with P. C. Mangelsdorf. 1961. Races of maize in Peru: Their origins, evolution and classification. Publication 915, National Academy of Sciences-National Research Council, Washington, DC, USA.
- Lacaze, X. and P. Roumet. 2004. Environment characterization for the interpretation of environmental effect and genotype  $\times$  environment interaction. *Theoretical and Applied Genetics* 109:1632–1640.
- Ministerio de Agricultura – Instituto Nacional de Recursos Naturales (MINAG-INRENA). 1995. Mapa Ecologico del Peru: Guia explicativa. MINAG-INRENA. Lima, Peru.
- Narro, L.A., S. Pandey, J.C. Perez, T. Calure, G. Eyherabide, M.X. Santos, S. Reyes, J. Celis and A. Navas. 1996. Incidencia de la sequia en la produccion de maiz en Sudamerica y la necesidad de un trabajo colaborativo. Pp. 7–13 *in* Proceedings of a Symposium on Developing Drought- and Low N-tolerant Maize, March 25–29, 1996 (G.O. Edmeades, M. Banzinger, H.R. Mickelson, and C.B. Peña-Valdivia, eds.). CIMMYT, El Batan, Mexico.
- Paterniani, E. and M.M. Goodman. 1977. Races of maize in Brazil and adjacent areas. *Centro Internacional de Mejoramiento de Maíz y Trigo. México, D.F.* 95 p.
- Sahagun C., J. 1998. Construcción y análisis de los modelos fijos, aleatorios y mixtos. *Boletín Técnico # 2. Departamento de Fitotecnia, Universidad Autónoma Chapingo.* 64 p.

- Sanchez G., J.J., M.M. Goodman and C. W. Stuber. 2000. Isozymatic and morphological diversity in the races of maize of Mexico. *Economic Botany* 54:43–59.
- Sari-Gorla, M., P. Krajewski, N. Di Fonzo, M. Villa and C. Frova. 1999. Genetic analysis of drought tolerance in maize by molecular markers. II. Plant height and flowering. *Theoretical and Applied Genetics* 99:289–295.
- SAS. 2000. The SAS System for Windows Version 8.1. SAS Institute, Inc., Cary, NC, USA.

## Synthesis – Drought

The reports on drought stress from Morocco, Burkina Faso, Mali, Peru, Zimbabwe, Zambia and Malawi address the characterizing of drought stress, the plant's response to low-water conditions, and understanding the interaction between farmers' strategies for drought and the genetic diversity for drought tolerance. Experiences from arid regions of Africa sharpen the difference between 'drought' *per se* and seasonal shifts in water conditions in arid climates. Climate data suggest that drought has become more extreme over the last half-century, but it is uncertain whether these conditions are part of a long-term, cyclic shift or true climate change. However, regardless of the cyclic or non-cyclic nature of changing climate patterns, these shifts will influence crop genetic diversity and farmers' crop maintenance strategies.

The reports from Mali and Burkina Faso examine plant traits that contribute to drought tolerance, in particular flowering time, time to maturity, and ability to maintain some level of yield under low-water conditions. Observations of drought-tolerant okra from Burkina Faso suggested that cell membrane permeability may also play a role in withstanding low-water conditions. Similarly, in Mali photoperiod sensitivity controls flowering date in some Guinea-race sorghums, thereby affecting drought susceptibility.

Farmer strategies to manage drought correlate with plants' tolerance characteristics. In Zimbabwe and Mexico, farmers plant combinations of early and late-flowering varieties in order to manage the risk of a mid-season drought, and farmer selection plays a strong role in the maintenance of distinctive varieties with defined flowering dates. In Burkina Faso, some farmers predict drought based on ecological characteristics of the native vegetation, suggesting that the intactness of the surrounding ecosystem may be crucial to farmers' planting choices. These observations not only highlight the importance of agromorphological and ecological markers to farmer seed selection, but also that farmers' criteria may differ from those of formal breeders for selecting the 'ideal' crop type.

## IV. Salt Stress

### On-farm conservation of rice genetic diversity under salinity stress: case study in a lowland agrosystem of Vietnam

*Nguyen Thi Ngoc Hue and In situ Project staff*

#### **Introduction**

Rice (*Oryza sativa*) has tremendous landrace diversity in Vietnam, where the landraces are adapted to particular geographic and climatic situations. Before the 1970s—when modern varieties (MVs) were introduced—all cultivars were of local origin. Thus a high level of rice diversity has been maintained because farmers use a diverse set of varieties as a strategy to cope with land heterogeneity and adverse conditions. In recent years, with access to technologically advanced seed, the use of improved varieties with higher yields has needed intensive farming, which has gradually reduced the area committed to local rice varieties, which have low productivity and economic returns. To ensure that local varieties are maintained, research on conservation of traditional rice genetic resource in particular and indigenous crops in general have been pressing issues.

On-farm conservation of rice diversity, an *in situ* type of conservation, refers to maintaining landraces in the farmers' fields. For on-farm conservation, the role of farmers is fundamental because they cultivate landraces for multiple uses, market preferences, disease resistance, suitable planting time, abiotic stress tolerance and for worship purposes (Nguyen Tat Canh et al. 2004). On-farm crop diversity does not only result from individual practices but also from the collective actions of farming communities. Therefore, the maintenance of crop diversity and varieties on-farm must be advantageous to farmers (Bellon and Smale 1998). Farmers have been practising on-farm conservation since agriculture existed (Engels and Wood 1999). The farmers used crop genetic diversity to manage abiotic stress such as drought, salinity and cold in their cultivation environments. That is why to access diversity, its structure and changes in regards to drought, salinity soil and cold stress are important.

This paper presents the synthesized research results on the extent of rice diversity and farmers' good practices for managing salt stress in lowland areas of Nam Dinh province (Red River Delta), Thua Thien Hue (Central Coast) and Tra Vinh (Mekong Delta). The results are extracted from data of on-farm conservation studies conducted by the participating institutions in the national project on 'Strengthening the scientific basis of *in situ* conservation of agricultural biodiversity on-farm' supported by the International Plant Genetic Research Institute.

#### **Methods**

This paper synthesizes results from participatory studies conducted in three sites in the lowland rice agrosystems, and of secondary data available from other related studies.

#### **The studied ecosites in lowland rice ecosystems**

The field studies were conducted in three ecosites in the lowland rice agrosystems. Ecosites were selected in the locations where the farmers still maintained traditional rice varieties under saline and acid soil conditions during 1996–2002, when rice culture was substantially important for the local livelihood. The three ecosites were:

- Kien Thanh and Dong Lac, Nghia Hung district, Nam Dinh province, representing a lowland rice ecosystem in the Red River Delta in the north of Vietnam. Nghia Hung has some areas subject to slightly saline soil bordering the sea.

- Quang Thai of Quang Dien and Phu Da of Phu Vang district, Thua Thien Hue province, representative of coastal lowland rice ecosystem suffering from salinity
- Dai An, Tra Cu district, Tra Vinh province, representative of a lowland rice ecosystem in the Mekong Delta, which is characterized by a slightly acidic soil.

### **Participatory research methods used at three ecosites**

#### **The RRA and PRA Survey**

The Rapid Rural Appraisal (RRA) and Participatory Rural Appraisal (PRA) tools were used to list the number and cultivated area of rice cultivars during the baseline surveys conducted by the *in situ* conservation project partners and to analyze the genetic diversity within farmers' rice varieties in Vietnam over time.

#### **Baseline survey**

Household surveys (39–140, 45–95% of total households) were carried out in five villages of four districts to create a list of farmer-named rice diversity and basic socioeconomic characteristics of rice growers. Descriptive statistical analyses of all parameters were computed for each site and analysis of varietal diversity indices was made with the Simpson index (SI). The Simpson index for quantifying rice diversity was calculated using the following formula:

$$H' = 1 - \sum fi$$

where  $fi$  is the relative frequency proportion of the farmer-named variety found in category  $i$ .

From 1998 to 2002 the surveys were carried out at five villages of the three ecosites to list the farmer-named varieties and to collect the ethnobotanical information.

Consistency in farmer unit of diversity (FUD): the varietal diversity measured by local names will have no value if farmers are not consistent in naming the rice cultivars. Farmers' descriptors for each cultivar have been documented.

#### **The Community Biodiversity Register (CBR)**

CBR is a participatory approach to make an inventory of rice genetic resources and to enhance the knowledge base, empowering a farming community to manage the local biodiversity it maintains over generations. In 2002 and 2003 a community biodiversity register survey at village level sampled 30 farmer households (HH) per village for all sites.

*Diversity fairs, diversity blocks, and focus group discussions* were used to calculate the diversity indices. The agromorphological traits and molecular markers were used to identify the genetic variation among rice cultivars. Crop diversity fairs were organized regularly in various farms at all studied sites during the project implementation. These were community initiatives with facilitation and support provided by the project to promote seed flows and exchanges among the households and communities. The collection of rice seed samples of all rice varieties being used was also conducted for morphological studies.

*Socioeconomic surveys, intensive data plots technique* and knowledge acquisition methods were also conducted to record the farmers' perceptions of rice genetic resources conservation and utilization and farmers' perceptions on salt tolerance of rice.



## Results and discussion

### Assessment of rice genetic diversity

Rice cultivation in Vietnam is by traditional practices. The growth of rice production and socioeconomic development provide more and more pressures on rice diversity, causing loss of traditional cultivars. A survey carried out in 2001 by the Ministry of Agriculture and Rural Development (MARD) showed that the number of traditional varieties (TVs) in the whole country, though still remaining considerably high, was much lower than that of modern varieties (MVs). The area planted to rice TVs was also very small, accounting for 3.6–21.4% of the total rice-growing area (Table 1). The survey also indicated that most irrigated rice lands were planted to rice MVs. Vietnam has 2 million ha of alum earth and salinity soil, mainly distributed in the Red River Delta, Cuu Long River Delta and coastal central, where many new varieties have been introduced which are saline and acid tolerant, such as Tep lai, OM576 8, VN-91-10, Nhi uu 838, C71, OM 90-2, OM 269, V14 and N.219. However, a study by *In situ* Project staff showed that in the marginal rain-fed environments such as upland, coastal sandy and flood-prone areas, many rice TVs were maintained because of their various adaptation to climate, production, consumption and cultural value.

**Table 1. Number of rice cultivars and area of rice TVs in regions of Vietnam in the cropping year 2000–2001 (MARD 2002).**

| National region | Winter-spring (WS) crop |            |                             | Summer-autumn (SA) crop |            |                             |
|-----------------|-------------------------|------------|-----------------------------|-------------------------|------------|-----------------------------|
|                 | No. of MVs              | No. of TVs | TVs growing area in '000 ha | No. of MVs              | No. of TVs | TVs growing area in '000 ha |
| Northern        | 160                     | 38         | 41.9 (3.6) <sup>†</sup>     | 145                     | 73         | 161.2 (11.5)                |
| Central         | 116                     | 13         | 8.8 (4.2)                   | 126                     | 41         | 59.1 (21.4)                 |
| Southern        | 167                     | 21         | 136.8 (8.4)                 | 147                     | 27         | 73.3 (4.7)                  |
| Total           |                         |            | 187.5 (6.2)                 |                         |            | 293.6 (8.9)                 |

<sup>†</sup> Number in parenthesis is percentage of rice TV in the total rice-growing area.

Baseline survey data showed that a considerable number of rice landraces are continuously maintained in lowland rice agrosystems. There are 41 local rice cultivars cultivated in three sites where some soils are characterized as saline or acid. However, this diversity varied from village to village and this changes over time. For example, in Nghia Hung District in the Red River Delta, out of 10 farmer-named varieties, 4 were rice landraces. Similarly, the study results in Thua Thien Hue reported that the number of TVs being used in 2000 was 5–7, accounting for more than one-third of total number of rice cultivars. There were 15 traditional varieties (37.5% of total rice varieties) planted at Dai An village (Tra Cu, Tra Vinh). In particular, the salinity and acid tolerant Trang Tep variety was grown by many HHs and in a large area (20% of total rice area).

Study of the structural change of rice varieties cultivated in three sites showed that the general trend was for an increase in the total number of rice cultivars at the community landscape level. This was mainly due to the increase in number of MVs. Number of TVs decreased in the areas where number of TVs was higher before 1998 such as in Central Coast and Cuu Long Delta. The change in number of varieties in the two study ecosites in Thua Thien Hue was similar. The number of MVs did not change in both study areas (8 in Quang Thai, 12 in Phu Da). However, the number of TVs decreased in both study areas (from 6 to 5 in Quang Thai, 11 to 7 in Phu Da) (Table 2). The change in number of MVs and TVS in the two study ecosites of Nghia Hung (Nam Dinh) between 2000 and 2002 was not significant. However, in Dai An (Tra Vinh), the number of TVs decreased from 20 in 1998 to 14 in 2002, whereas the number of MVs increased from 9 (1998) to 25 (2002) due to increasing rice cropping seasons per year—farmers adopted more MVs for this practice.

Table 2 also illustrates the comparison between sites for rice diversity indices. The highest diversity index (evenness of rice) was found in Thua Thien Hue site, followed by Tra cu, and Nghia Hung. The findings on the changes in SI were consistent with the changes in number of varieties as well as the changes in variety abundance. The reduction of the number of traditional varieties and the lower evenness of the share in total rice area both contributed to the decrease in SI. It was also noted that most TVs were the rare varieties, which were grown by few households and in a small area. However, although the MVs were used with increasing frequency, some TVs were maintained as common varieties with large distribution. Among the common rice TVs being maintained in the lowland rice ecosystems were *Tam co ngong* (in Red River Delta), *Heo*, *Chum bat*, *Chien*, *Nuoc man* (in Central Coast), *Phi rang* and *Trang tep* (in Mekong Delta) due to their adaptation to saline and acid soils. Seed flow of traditional varieties in general and common landraces named in these communities was characteristic of an informant seed system. Our study indicated that 100% of traditional variety seed used by farmers in study sites is supplied by the informal system.

**Table 2. Number and type of rice cultivars in different ecosites and regions of Vietnam.**

| Province/<br>national region | Nam Dinh/Red River Delta |               |               |               | Thua Thien Hue/Central Coast |               |           |             | Tra Vinh/<br>Mekong Delta |        |
|------------------------------|--------------------------|---------------|---------------|---------------|------------------------------|---------------|-----------|-------------|---------------------------|--------|
|                              | Dong<br>Lac              | Nghia<br>Hung | Kien<br>Thanh | Nghia<br>Hung | Quang<br>Thai                | Quang<br>Dien | Phu<br>Da | Phu<br>Vang | Dai An                    | Tra Cu |
|                              | 2000                     | 2002          | 2000          | 2002          | 1996                         | 2001          | 1996      | 2001        | 1998                      | 2002   |
| Number of MVs                | 5                        | 14            | 9             | 11            | 8                            | 8             | 12        | 12          | 9                         | 25     |
| Number of TVs                | 4                        | 3             | 4             | 3             | 6                            | 5             | 11        | 7           | 20                        | 14     |
| Total cultivars              | 9                        | 17            | 13            | 14            | 15                           | 13            | 23        | 19          | 29                        | 39     |
| Simpson Index                | 0.73                     | 0.52          | 0.72          | 0.61          | 0.85                         | 0.50          | 0.79      | 0.65        | 0.69                      | 0.53   |

Source: Field data, Tuyen et al. 2004.

### Farmers' practices for managing salt stress

Farmers maintain a number of landraces since they value them highly for various production, consumption and other observable use characteristics. The current stock of rice landrace diversity is obviously the product of intricate heterogeneous agroecosystems operating under a number of socioeconomic constraints.

Among the conditions that have highest impact on farmers' rice diversity management is the level of irrigation. In the irrigated agrosystems farmers replace TVs with MVs to increase rice productivity (high yield and cropping rate). In the irrigated areas, the percentage of households who planted TVs was much lower than in the rain-fed lowlands (14.5 and 53.8%, respectively). Total number of rice cultivars per household in irrigated systems was higher than in rain-fed (2.3 and 1.8, respectively). However, number of rice TVs and TV growing area at household level was much higher in rain-fed systems than in the irrigated. It was found that the rice diversity is still maintained in rain-fed lowland rice systems, but is not completely lost in the irrigated one.

Among the abiotic stresses that have significant impact on rice diversity management of farmers in coastal and Mekong River Delta areas are the levels of salinity and acid tolerance of local varieties. In the process of determining the best varieties for their farms, farmers try all possible alternatives and only adopt those that have one or more relative advantage over the existing ones. In marginal conditions, farmers' tendency is to grow at least one traditional stable variety as a part of food security. Diversity is maintained as part of utilizing different types of soil fertility regimes and to support different uses.

The results of study in Nghia hung, Phu Vang and Tra Cu districts indicate that local people can distinguish crop varieties and know the relative values of each variety, so they can select the crop or varieties needed to meet their own demands. There, apart from salt stress tolerant rice, farmers still grow taro, which is considered to be a salt-tolerant crop. In coastal rice agrosystems, salinity and acidity tolerance are considered to be valuable traits among 10 different selection criteria given by farmers. It was found that in Quang Thai and Tracu—where there are some areas with saline soils—62–77% of households selected rice varieties for salinity tolerance. In these areas the farmers have good experiences in rice selection. The most straightforward plant breeding technique of farmers is mass selection. These are not necessarily static over time in the way they operate. The salinity-tolerant varieties such as Hau, Nuoc man, Chien Thai and Trang tep were selected and highly appreciated in these communities.

To select the best varieties to cope with salinity stress, the farmers usually observe and evaluate plant status in the fields. They consider that the critical stages of rice for salinity and acid stress are tillering and booting. In order to test and select good salt- and acid-tolerant varieties, farmers can use some feature such as colour and vigour of root system; colour of leaf and growth level when comparing the varieties in the field when the plants are at the stages of 30 days after planting and at booting (Table 3).

**Table 3. Farmers' evaluation criteria for salt and acid stress tolerance of rice.**

| Parts of plant | Salinity and acid-tolerant variety | Salinity and acid-susceptible variety |
|----------------|------------------------------------|---------------------------------------|
| Root system    | White colour and emerged rootlets  | Black colour and no rootlets          |
| Leaves         | Green and developed                | Yellow–brown and dies                 |
| Plant growth   | Good growth and tillering          | Poor growth and tillering             |

The results of PRA and group discussions showed that the farmers, through their practices and experiences, can classify the rice varieties into groups that relate to tolerance level to salt stress in rice cultivation conditions (Table 4). In such sites the farmers chose the rice landraces but not MVs for managing salinity stress. The impact of the 1999 catastrophic flood in Thua Thien Hue (also called the Century Flood) served as an example. The rainfall in November reached an unusual 2500 mm, which caused the flood that damaged seeds and caused loss of varieties of most farmer households in the region. All the farmers interviewed were willing to again have traditional varieties such as *Chien thai*, *Nuoc man* and *Chum muon* to grow because of their high tolerance of salinity stress and poor soil.

**Table 4. Rice variety arrangement according to salt stress tolerance.**

| Low salinity and alum earth tolerance  | Medium salinity and alum stress tolerance      | Good salinity and alum stress tolerance   | High salinity stress tolerance  |
|--|--|---|---------------------------------|
| Tam xoan, Nep cai hoa vang, Nep thai binh, Troi bien, Keo siem, Phi trang, Lem lun, Trang lun, Tam ran, Ngang co | Heo, Heo chum, Chum dau, Phi rang, Nep ba lao, | Hau, Nep ong lao, Tam co ngong, Trang tep | Chien thai, Nuoc man, Chum muon |

To minimize damage of salt stress, the farmers not only know how to use varietal diversity to cope with salinity stress but also to apply good practices for plant protection. Farmers' perception is that before growing rice seedlings, the following process (in Vietnamese, *thau chua* and *rua man*) should be carried out:

- First, the field is flooded with sweet water (from river water or rain-fed)
- Second, the soil is ploughed under flooding; which effectively drains the saline water from the fields
- The field is again watered and seeds are sown or seedlings are transplanted.

In this way it is believed that the level of iron ( $\text{Fe}^{++}$  and  $\text{Fe}^{+++}$ ) and aluminium ( $\text{Al}^{+++}$ ) toxicity are decreased and rice can grow and develop better.

From above results and discussion we can make some recommendations:

- Irrigation improvement was the key factor that favoured the replacement of TVs with MVs. The force behind this practice was the economic returns. The measures adding values to the traditional varieties and irrigation should be integrated to support on-farm conservation.
- Farmer's good practices to cope with salinity stress resulted in on-farm conservation of rice diversity, but this was not the farmers' objective. Support for awareness and capacity-building for the concerned stakeholders is critical at the start of any conservation programme.

The research results show that for a typical crop of marginal environments of northern, central and southern areas of Vietnam, where salinity and acid tolerant rice is required, it is possible to exploit genetic differences for these specific tolerances with low additional input and some improved techniques such as increased density of planting and improvement of fertilization methods. Breeding for specific adaptation such as salt stress not only offers a solution to how to improve agricultural production in marginal environments, but also provides a means of increasing production in a sustainable way.

The salinity and acid soil tolerant landraces such as *Trang tep*, *Chum muon*, *Chien thai*, *Nuoc man*, *Tam co ngong* and *Nep ong lao* should be used for PPB or PVS to provide more benefit to farmers in communities affected by these stresses.

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### **References**

- Bellon M.R. and M. Smale. 1998. A conceptual framework for Valuing On farm Genetic Resources. Economics Program Working Paper. Draft, CIMMYT, Mexico.
- Engels, J.M.M. and D. Wood. 1999. Conservation of agrobiodiversity. Pp. 355–386 in *Agrobiodiversity characterisation, utilisation and management* (D. Wood and J.M. Lenne, eds.). Agrobiodiversity International, CABI Publishing, Milnthorpe, UK.
- Nguyen Tat Canh, Nguyen Thi Ngoc Hue, Pham Van Hien et al. 2004. Scientific basic of on-farm conservation for upland rice diversity: Key issue for consideration of policy development in Vietnam. Pp. 23–39 in *On-farm management of agricultural biodiversity, lesson learnt and policy implications. The Proceedings of National Symposium 28 March–1 April 2004, Hanoi, Vietnam. Publishing House of Agriculture, Hanoi. [In Vietnamese]*.
- Truong Van Tuyen, Nguyen Vinh Truong, Hoang Thi Thai Hoa, Le Thieu Ky et al. 2004. Farmers' practice of conservation of rice diversity on farm: Lesson learnt and policy implication in coastal lowland rice agrosystem. Pp. 40–59 in *On-farm management of agricultural biodiversity, lesson learnt and policy implications. The Proceedings of National Symposium 28 March–1 April 2004, Hanoi, Vietnam. Publishing House of Agriculture, Hanoi. [In Vietnamese]*.

## Indigenous knowledge in management of abiotic stress: Date palm genetic resources diversity in the oases of Maghreb region

*Abdelmajid Rhouma, Nouredine Nasr, Abdelmalek Zirari and Malek Belguedj*

### Introduction

Date palm (*Phoenix dactylifera*) is the main crop cultivated in the Maghreb oases. In the oases several fruit species are planted under date palms; their diversity and their importance vary from one oasis to another: olive trees, fig trees, pomegranate trees, citrus, apricot tree, banana tree, etc. Underneath the date palms and fruit trees, several vegetable species, forage crops and commercial species such as henna and tobacco are often cultivated (Rhouma et al. 2003, 2004).

Genetic resources cultivated in the oases have been the object of selection for centuries and millennia of experimentations and farming selections. This selection is oriented to reply to the needs of oasis communities but is dictated by several abiotic stresses.

The team of UNDP-GEF, RAB98/G31 project 'Participatory management of genetic resources of date palm in the oases of the Maghreb region (2001–2005)', executed by IPGRI and INRA of Algeria, Morocco and Tunisia, tries to preserve, *in situ*, diversity of date palm ([www.maghrebdatepalm.org](http://www.maghrebdatepalm.org)).

The oases of these three countries cover an area of approximately 181 000 ha comprising 21 million date palms and producing around 432 000 t of dates. In these oases, several cultivars and *khalt* or *Sayer*<sup>2</sup> of date palm are threatened with disappearance owing to different biotic, abiotic and socioeconomic factors (IPGRI et al. 2004, 2005).

In this paper we present summaries of the indigenous knowledge of farmers of five project sites (Figure 1) and related to abiotic stress and management of date palm genetic resources diversity.

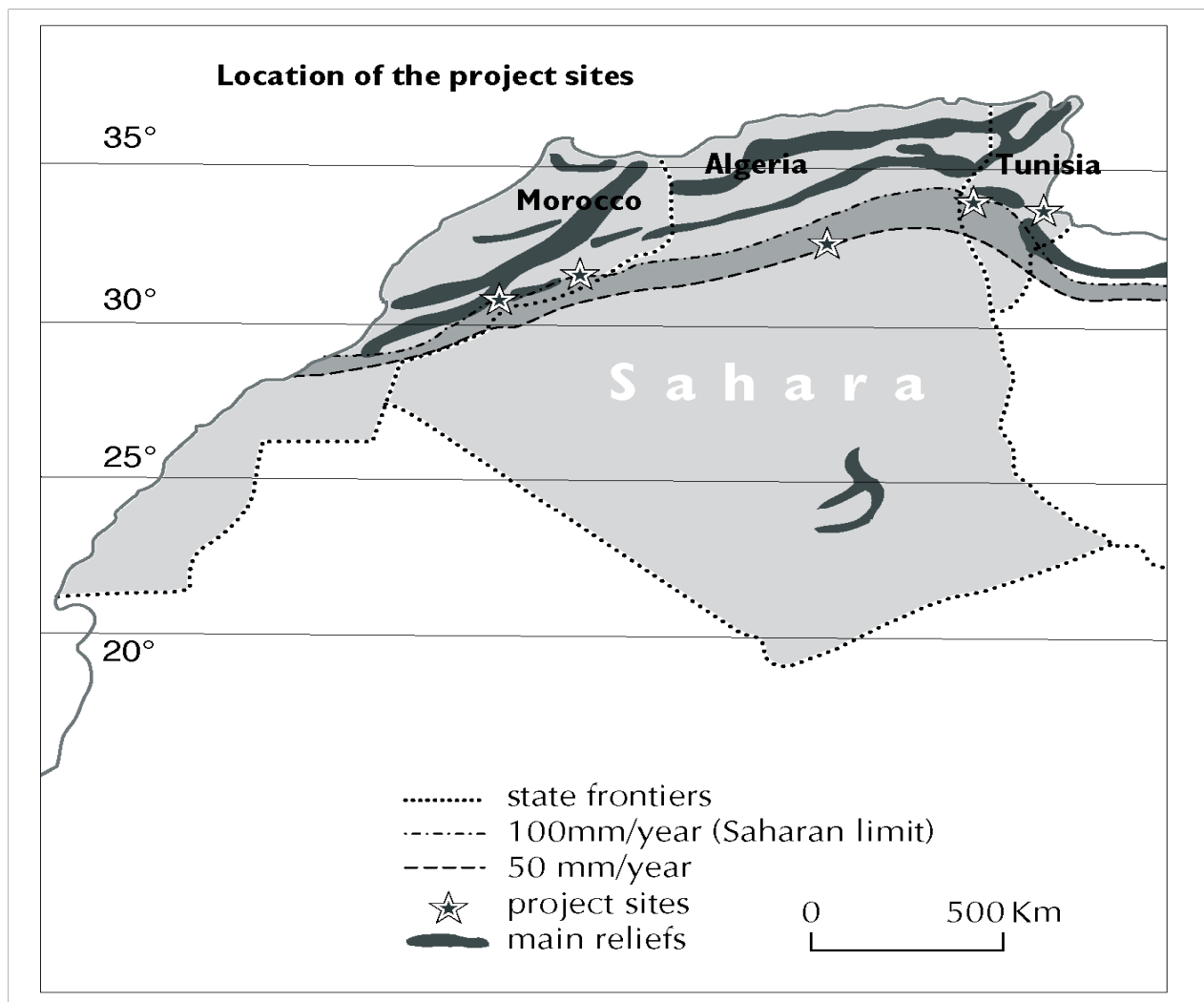
### Methods

Five aspects affect the decision-making of the farmer and allocation of plant diversity: What agromorphological characteristics are to be selected? What exploitation practices are to be used? Where to plant? What dimensions of the population to plant? And what are the seed sources? (Jarvis and Hodgkin 2000).

Results presented in this article are collected from:

- About 20 participatory rural appraisals (PRA) in the project sites led by project teams.
- Different workshops organized in the project sites with groups of Maghreb farmers during national and regional study tours of farmers.
- Workshops organized with farmers in the project sites for the inventory of indigenous knowledge of date palm genetic resources management.

<sup>2</sup> A palm tree grown from seed that has not been the subject of large multiplication by vegetative propagation, and whose geographical distribution is thus limited.



**Figure 1. Location of Maghreb date palm project sites (left to right): Fezouata and Aoufous in Morocco, Ghardaia in Algeria, Degache and Chénini-Gabès in Tunisia.**

## Results

In oasis ecosystems that have remained isolated for several centuries in the very hostile desert environment, farmers have selected the cultivars of dates responding to their vital needs. They have selected a range of cultivars of with a maturity spread covering an important part of the year (end of June to January; Table 1) so that they can obtain fresh fruit as early as possible from the precocious cultivars, and as late as possible from the late cultivars. They have also selected cultivars that can be preserved, to stock them and consume the dates the longest possible after the harvest (Benkhalifa et al. 2003; Martin et al. 2003; Nasr 2004; IPGRI et al. 2002, 2003).

However, biotic and abiotic stresses often limit the choice of farmers, influence the selection and impel farmers to choose cultivars resistant and/or tolerant to specific stress. In the Maghreb oases, these factors, often very severe, are therefore considered as abiotic stress for cultivated species (prolonged drought, excessive heat, violent and sandy winds, very irregular and often torrential rain, mediocre quality of water and soil, frequent flooding).

**Table 1. Periods of maturity of some main date palm cultivars of the region.**

| Cultivar             | June | July | Aug. | Sept. | Oct. | Nov. | Dec. | Jan. |
|----------------------|------|------|------|-------|------|------|------|------|
| Cheikh Mhammed (2)   |      |      |      |       |      |      |      |      |
| Tamezwert (2)        |      |      |      |       |      |      |      |      |
| Baydir (2)           |      |      |      |       |      |      |      |      |
| Ahardane (4)         |      |      |      |       |      |      |      |      |
| Aguellid (4)         |      |      |      |       |      |      |      |      |
| Boufeggous (4)       |      |      |      |       |      |      |      |      |
| Ammari (3)           |      |      |      |       |      |      |      |      |
| Goundi (1)           |      |      |      |       |      |      |      |      |
| Ghars (3)            |      |      |      |       |      |      |      |      |
| Besser Helou (1)     |      |      |      |       |      |      |      |      |
| Lagou (1)            |      |      |      |       |      |      |      |      |
| Bent Q'Bala (2)      |      |      |      |       |      |      |      |      |
| Mejhoul (4)          |      |      |      |       |      |      |      |      |
| Bouskri (4)          |      |      |      |       |      |      |      |      |
| Jihel (4)            |      |      |      |       |      |      |      |      |
| Deglet Nour (1) (2)  |      |      |      |       |      |      |      |      |
| Aligue (1)           |      |      |      |       |      |      |      |      |
| Khalt Chetoui (1)    |      |      |      |       |      |      |      |      |
| Akerbouch (2)        |      |      |      |       |      |      |      |      |
| Tissibi (2)          |      |      |      |       |      |      |      |      |
| Bousthammi-Kahla (4) |      |      |      |       |      |      |      |      |
| Iklane (4)           |      |      |      |       |      |      |      |      |
| Ras Ltmar (4)        |      |      |      |       |      |      |      |      |

(1) Tunisian cvs., (2) Algerian cvs., (3) Cvs. in common between Algeria and Tunisia, (4) Moroccan cvs.

### Drought stress

The climate of the Maghreb oases region is extremely arid. Farmers, in such a situation, choose cultivars of date palm whose dates are of soft consistency, with early maturity and can be harvested sequentially as they ripen (*grappillage*) and/or harvested at the 'besser' stage (stage of development of the date where the fruit has its complete form and normal size, generally red or yellow colour, sometimes greenish) to avoid critical periods of drought, such as: Ammari, Lagou, Gasbi, Besser-Helou and all the precocious *khalt*s of soft dates). In the project sites, some cultivars are tolerant to drought (such as Azerza, Akerbouch, Kentichi/Mech-Degla, Bousthammi-Kahla, Iklane, Bouzeggagh) and are appreciated by farmers. In some oases where the production of date palm is secondary to that of other crops, date palm is cultivated especially to create a favourable microclimate for sensitive species: vegetables, pomegranate, fig trees, citrus, orange tree, banana, etc. (Benkhalifa et al. 2003, Rhouma et al. 2003, 2004).

### Important relative humidity and rain stresses

Dates are very vulnerable to the rain and to excessive humidity of the air, during some stages of their development. In regions where this phenomenon is usual (for example, coastal and insular oases in Tunisia: Djerba, Gabès) or in regions of risk (autumn rains), farmers have selected the cultivars that during these critical stages escape this stress. The farmers have thus selected the cultivars in response to the climate and its impacts (Benkhalifa et al. 2003; IPGRI et al. 2002, 2003; Martin et al. 2003; Rhouma et al. 2003, 2004, Tirichine and Benkhalifa 2002; Zirari 2002):

- Cultivars of precocious maturity: Goundi, group of Cheken (equivalent of *khalt* but of soft dates and generally harvested by *grappillage*), Gasbi, Lagou, Aherdane, Aguellid, Boufeggous, etc.

- The precocious cultivars such as Baydir, Ammari, Cheikh Mhammed, and even Ghars are particularly sensitive to the humidity and especially to rain. Because of their precocity, they are saved from autumnal rains.
- Cultivars harvested at a precocious stage before complete maturity (Besser-Helou, Arechti, Matata, Lemsi, Bourar, Jihel, etc.)
- Tolerant cultivars: Kenta, group of Khalt of dry fruit, Aguiwa, Boucerdoune, Garn-Ghazel, Degla-Baidha, Kentici/Mech-Degla, etc., that endure autumnal rains without damages.

Other cultivars are equally sensitive, such as Deglet-Nour, Dalat, Bent'-Qbala, Timdjouhart, Azeza, Tissibi, Sebaa-Bedraa, Tafezouine, Aligue, Bidh-Hamem, Mejhoul, Oum-N'hall, Mekt and Asaddam, but only if rains are frequent, which raises humidity in the oasis. Fruits of the cultivars Timdjouhart and Kaci-Moussa deteriorate after the first rains.

### **Violent wind stress**

Date palm, with a very powerful root system and a slender silhouette, is one of the fruit species most resistant to winds (Al Beker 1972). It is often used, among others, to protect sensitive species from wind (legumes, banana, orange tree, citrus tree, etc.). Wind has, however, an ominous influence on date palm, particularly at the season of pollination (no fertilization and fall of flowers) and at the maturation of fruit (fall of fruits). Some cultivars have, even at this level, a relative resistance and are the most often planted all around the farm such as: cultivars Kentichi/Mech-Degla, pollinators, Khalts, etc.).

### **Excessive heat stress**

Heat is a necessity for the maturation of dates. Date palm resists very strong heat. Excessive heat over a long period and during stages of fruit development (*besser*, *rotab* and *tamer*) can, however, influence the quality of dates of some cultivars of soft consistency and half-soft consistency. Fruit are thereby of small size, rounded and dry. In such a situation, farmers choose precocious cultivars to limit the period of heat influence (Aguellid, Aherdane), the cultivars harvested by *grapillage* to collect them bit by bit according to their maturity (Mejhoul, Boufeggous) and the cultivars of dry consistency and half-dry consistency such as Ammari, Om-Laghez, Gasbi, Tezerzit Soda, Kentichi/Mech-Degla, Tarzawa, Bousekri, Jihel, etc.

### **Cold stress**

Unlike heat, very low temperatures are rather ominous for the date palm and its production. The adult date palm can withstand low temperatures ( $-7^{\circ}\text{C}$ ) and even if green palms die because of low temperatures the terminal bud, well protected and influenced by temperatures of the soil (irrigation water) and the ambient air, can regenerate after the cold wave. Abrupt changes can cause severe damages as during 2004–2005 when the winter was relatively rigorous in northern regions of Algeria and some southern oases of Morocco. In Setif (Algeria), the date palms that adorned the city and its periphery all withered because the temperature was  $-14^{\circ}\text{C}$  early in 2005. In Errachidia (Morocco), date palms were severely affected in several places where temperatures of around  $-12^{\circ}\text{C}$  were recorded in December 2004.

### **Poor soil stress**

Most oasis soil is poor in structure and organic matter. Farmers are aware of this great constraint and know that for good production of dates, date palms need light deep soil with good drainage. Farmers place organic manure in the second most important position after irrigation water, and regularly add substantial quantities of organic manure. However, in situations where soil can not be corrected, farmers usually choose cultivars tolerant/adapted



to poor soils, which give acceptable production in both quantity and quality (e.g. Kentichi/Mech-Degla, Kenta, group of Khalt, Sayer Layalat, Jihel, Bousthammi-Kahla, etc.).

### Stress linked to waterlogging

In some situations, where plots are frequently filled with water following a rise of the water table in an oasis or because of bad drainage, farmers plant cultivars of date palm adapted to these soils, such as Besser-Helou, Kenta, Hachen El-Hamma, etc.).

### Salinity stress

Salinity affects the date palm as follows:

- The vegetative aspect of the tree changes, as it begins exhibit yellowish colours when the salinity of water exceeds 14–15 g of salt/L. This is visible on trees situated near a drainage canal, for example, and concerns all the cultivars.
- The influence of salinity is more pronounced on the cultivars of half-soft dates (Deglet-Nour, Akerboucht, Babati, U'rus, Menakher, Aligue, Mejhoul, Boufeggous, etc.). The fruit quality is therefore decreased and dates become more dry and wrinkled. In contrast, the cultivars of soft dates are not only more tolerant but their taste improves (sweeter) compared with those irrigated with sweet water (e.g. Kentichi/Mech-Degla, Degla-Baidha, Ammari, Besser-Helou). These cultivars give very good yields in the valley of Oued-Righ (Algeria), known as a very salty zone (for Degla-Baidha the average yield would exceed 150 kg/tree). Another very significant example: farmers who do not have access to public and good water for irrigation in the oasis of Degache dig wells to pump from the water table. Farmers call these wells *Aouinet* (small springs) where they have planted these tolerant cultivars and a group of Khalt and Cheken cultivars that currently constitute the genetic wealth of the oasis of Degache.

**Table 2. Resistance/tolerance of date palm cultivars to abiotic stress (main cultivars of the region).**

| Cultivar                | Stress  |                       |              |           |          |          |
|-------------------------|---------|-----------------------|--------------|-----------|----------|----------|
|                         | Dryness | Rain and air humidity | Violent wind | Poor soil | Salinity | Flooding |
| Besser Helou (1)        | +       | +                     | –            | +         | +        | –        |
| Kentichi/Mech–Degla (3) | +       | +                     | +            | +         | +        | +        |
| Ammari (3)              |         | +                     | –            | +         |          |          |
| Kenta (1)               | –       | +                     | –            | +         | +        | +        |
| Timjuhart (2)           | +       | +                     | +            | +         | +        | +        |
| Bent Qbala (2)          | +       | –                     | +            | +         | +        | +        |
| Ghars (3)               | +       | –                     | +            | +         | +        | +        |
| Bousthammi N (4)        | +       | +                     | +            | +         | +        | +        |
| Iklane (4)              | +       | +                     | +            | +         | +        | +        |
| Bouzeeggagh (4)         | +       | +                     | +            | +         | +        | +        |
| Jihel (4)               | +       | –                     | +            | +         | +        | +        |
| Mejhoul (4)             | +       | –                     | +            | –         | –        | +        |
| Boufeggous (4)          | +       | –                     | +            | –         | –        | +        |
| Deglet Nour (3)         | –       | –                     | –            | –         | –        | –        |
| Male date palm          | +       | +                     | +            | +         | +        | +        |

(1) Tunisian cvs., (2) Algerian cvs., (3) Cvs. in common between Algeria and Tunisia, (4) Moroccan cvs.

These abiotic stresses have, however, an influence on the production but that is very variable in function of the cultivars. Some cultivars have a site-specific adaptation with strict edaphic demands and their production is strongly influenced qualitatively and quantitatively by abiotic stresses. These are unfortunately the commercial varieties: Deglet Nour in Tunisia and Algeria, Mejhoul and Boufeggous in Morocco, and other less known cultivars. However, other cultivars have flexible adaptation and tolerate relatively important variations without negative influence on their production, such as Kenta, Besser-Helou,

Ammari, Black Bousthammi-Kahla, Iklane, Tademamt and others (Benkhalifa et al. 2003; IPGRI et al. 2002, 2003; Martin et al. 2003; Rhouma et al. 2002, 2004, Tirichine and Benkhalifa 2002; Zirari 2002).

### **Discussion and conclusions**

In the oases of Maghreb Region, date palm is usually cultivated for its products and by-products but also to protect underlying crops. Date palm is one of the rare species that tolerates or resists extreme abiotic stress. Al Beker (1972) reports that there is no place in the world where the date palm can not tolerate strong heat.

Abiotic factors, which are very severe in the oases of the Maghreb, are variable in space and time, which limits the geographical distribution area of the cultivars of specific adaptations. Nevertheless, date palm offers very rich genetic resources diversity, able to adapt to different situations and to add value to natural resources sometimes limited in quantity and in quality. Farmers have the indigenous knowledge to utilize this diversity to overcome the abiotic stress of each situation.

Maintaining the sustainability of an oasis ecosystem is possible only with date palm with its tremendous genetic wealth and with the farmers, who are aware and knowledgeable of the rigours of abiotic stress and how to master them with good management of genetic resources. Research in this sense is to be developed to inventory and analyze and to build on indigenous knowledge. It is particularly recommended to:

- Study abiotic factors dominant at regional levels, as well as trends of their variation over time.
- Encourage scientific research that aims to determine physiological tolerance mechanisms and resistance of cultivars to the various abiotic stresses.
- Determine, in each region, the cultivars with flexible adaptation that are resistant to abiotic stresses.
- Encourage the multiplication and exchange of the germplasm of these cultivars between farmers on the basis of abiotic factors of each region.

### **References**

- Al Beker, A. 1972. The date palm: a review of its past and present status, and the recent advances in its culture, industry and trade. El Waten edition, Iraq. 1085 p.
- Benkhalifa, A., M. Guerradi, A. Tirichine, M. Belguedj and L. Labгаа. 2003. Diagnostic des palmeraies : El atteuf, Metlili, Berriane, Beni Isguen, Guerrara, Mélika, Ghardaïa, Boulila, Daya, Mansoura, Zelfana, Sebseb, Bounoura. IPGRI et INRA, Algérie. 400 p.
- IPGRI, ICRA and INRAT. 2002. Sauvegarde de la diversité génétique du palmier dattier dans l'oasis de Degache (Tunisie) ; problèmes et perspectives. Série documents de travail de l'ICRA n° 107. Rapport du projet PNUD-FEM, RAB98/G31. 89 p.
- IPGRI, ICRA and INRAT. 2003. Valorisation de savoirs et savoir-faire : Perspectives d'implication des acteurs, dont la femme dans la conservation *in-situ* de la biodiversité du palmier dattier dans les oasis du Djérid (Tunisie). Série documents de travail de l'ICRA n° 115. Rapport du projet PNUD-FEM, RAB98/G31. 96 p.
- IPGRI, INRA Tunisia, INRA Algeria and INRA Morocco. 2004. Market study of date palm in the Maghreb région; Final report. Report of UNDP-GEF, RAB98/G31 project with UNOPS support. 40 p. and annexes: summaries of national studies and investment and development projects.
- IPGRI, INRAA, INRAM, INRAT, FEM et PNUD. 2005. Descripteurs du Palmier dattier (*Phoenix dactylifera* L.). Institut International des ressources phylogénétiques, Rome, Italie ; Fonds pour l'Environnement Mondial, Washington, Etats-Unis, Programme des nations Unies pour le Développement, New York, Etats-Unis ; Institut National de la recherche Agronomique, d'Algérie, du Maroc et de Tunisie. 71 p.
- Jarvis, D. and T. Hodgkin. 2000. Farmer decision-making and genetic diversity: linking multidisciplinary research to implementation on-farm. Pp. 261–278 *in* Genes in the Field. On-farm Conservation of Crop Diversity (S. Brush, ed.). IDRC/IPGRI/Lewis Publishers.
- Martin, G., A. Bouaziz, O. Benlahbib, A. Zirari, P. Eyzaguirre and S. Barrows. 2003. Etude de la diversité génétique du palmier dattier dans les oasis de Aoufous en employant les méthodes participatives. IPGRI CWANA Newsletter 25:11–16 [in Arabic].

- Nasr, N. 2004. Importance de la diversité génétique du palmier dattier dans la conservation des oasis et l'amélioration des conditions de vie des populations oasiennes au Maghreb. Atelier régional pour la 'La Protection Intégrée (IPM) du Palmier Dattier dans les pays de l'Afrique du Nord'. FAO-IRESA Tunisie, Tozeur (Tunisie) : 11-13 Décembre 2003. 7 p.
- Rhouma, A., M. Ben Salah et N. Nasr. 2004. Diagnostic participatif pour la réhabilitation de la diversité génétique du palmier dattier dans l'oasis de Chénini. 56 pages and annexes [in Arabic].
- Rhouma, A., N. Nasr, A. Ben Ali et N. Hamdouni. 2003. Diagnostic participatif sur la diversité génétique phoenicicole et la situation socio-économique dans l'oasis de Hammet El-Jérid (Tunisie). IPGRI, INRAT, ACT, AID, GID and CRDA, Tozeur. 63 p.
- Tirichine, A. et A. Benkhalifa. 2002. Impact du marché sur la conservation de la biodiversité : cas des cultivars de dattes dans la région de MZAB en Algérie. Séminaire international sur le développement de l'agriculture saharienne comme alternative aux ressources épuisables. Biskra, 22-23 Octobre 2002. IPGRI et INRAA. 10 p.
- Zirari, A. 2002. Date palm cultivation in the Drâa valley, Morocco: Its potentials and constraints. IPGRI CWANA Newsletter 24:5-6.

## **Synthesis – Salinity**

Results from studies of varietal diversity and salinity from Vietnam and Tunisia expand on the complex relationship between genetic mechanisms for salt tolerance, market-oriented production systems and broader social conditions, suggesting that both these factors and the biological basis for salt tolerance need to be further explored. In both countries, salinity-tolerant varieties were planted on marginal lands, which were neither entirely outside of the formal market system nor outside large-scale, monoculture practices. Government policies were sometimes adverse for the continued use of such crops. In some countries, government-promoted crops are frequently not useful to farmers in these marginal areas. In this vein, it was observed that in Tunisia the two date palm varieties most frequently promoted by the government are known to be very susceptible to salt stress. On the other hand, many farmers know of and use saline resistant cultivars of date palm in Tunisia, and rice in Vietnam, particularly in areas subject to salt stress. The stress may represent a significant agent of genetic erosion. These stress situations also place a premium on maintaining the seed supply of tolerant traditional varieties, e.g. after serious widespread flooding.

The research questions on the genetic structure of populations that can survive high salt stress include the following: Is yield stability in patchy saline soils achieved by populations with low diversity and a high frequency of tolerant genotypes, or by high diversity with low frequency of tolerant genotypes? If the latter is the case, is it likely that only farmers from salt-stressed environments will have crops with genes for salt tolerance? How often is tolerant diversity latent in populations nearby or away from areas experiencing this stress?

## V. Cold Stress

### Improvement of maize chilling tolerance by breeding

*Csaba L. Marton*

#### **Introduction**

Maize genotypes which are cultivated in cooler climates or at higher latitudes require a short vegetation period and adaptation to low temperatures. Real adaptation to low temperature seems to be rather complex. It requires a number of features: resistance to chilling, resistance to soil fungi during germination and the ability to grow at low temperatures.

Since the optimum temperature for maize is around 30°C, a broad temperature range is covered by the term 'low temperature' when interpreting chilling tolerance. Three ranges are generally distinguished in the literature: Freezing (below 0°C), Low, non-freezing (0–6°C) and Suboptimal (6–20°C).

Maize is sensitive to frost in all phases of its growth cycle except as dry seed. Genetic variation seems to be very small, so tolerance to freezing temperature cannot be successfully improved by breeding. Freezing injury limits the expansion of the maize growing area.

Low non-freezing temperatures (0–6°C) cause physiological damage to the seedlings. Wilting and discolouration of the leaves are symptoms of chilling injury. With severe chilling, plants, or plant parts, are killed. The dysfunction of membranes at low temperatures could be the primary cause of chilling injury. The severity of the damage depends on the temperature, the duration of the cold treatment, the stage of development, and to a certain extent on the genotype. In practice, however, maize is sown in spring, when soil temperatures are seldom below 6°C for long periods of time, and it seems therefore unlikely that survival and emergence are affected by chilling.

The temperature during emergence and seedling stages frequently falls in the suboptimal range. These temperatures generally are not detrimental to seed or seedling, but do retard significantly the growth and development. Therefore breeders have long attempted to improve the chilling tolerance of maize.

By growing cold-tolerant hybrids it is possible to achieve an improvement in stand uniformity, the rate of plant development and the yield. In addition, cold-tolerant hybrids can be sown earlier, so hybrids with longer vegetation periods and greater yield potential can be grown. According to Berzsenyi et al. (1999) earlier planting results in higher yield with lower moisture content.

Field selection is an effective way of improving chilling tolerance, but depends greatly on the climatic factors of the year and growing site, so it is very uncertain. To avoid this problem breeders have elaborated laboratory cold test methods. In addition to the 'cold test' (Tatum 1942; Isely 1950; Clark 1954) numerous other methods have been developed to test the vigour of seeds under low-temperature conditions: Rolled towel method (Hoppe 1955), Accelerated aging test (Delouche-Baskin 1973), Tetrazolium test (Moore 1962), Seedling growth rate (Burriss et al. 1969), Pericarp injury test (Koehler 1957), Electric conductivity test (Matthews and Bradnock 1968) and Complex stressing vigour test (Szirtes et al. 1982).

The main aim in the present studies was to evaluate genetically determined characters, especially the genetic background of seedling chilling tolerance, which has been less widely studied.

### Materials and methods

The examinations were made on eight unrelated inbred lines with different agronomic properties. The chilling tolerance of the lines exhibited considerable differences in early studies. The lines were crossed in all possible combinations, including reciprocals, according to a diallel scheme. Depending on the size of the phytotron unit, the complete diallel or only the lines were tested.

It was hoped to determine whether there was any relationship between chilling tolerance at emergence and at the seedling stage, what forms seedling chilling tolerance could take, and what genetic factors were involved in this.

Investigations were made on the effect of the *Fusarium* species prevalent in Hungary (*F. oxysporum*, *F. culmorum*, *F. graminearum*, *F. poae*) on the development of chilling tolerance, while the effect of the environment (low temperature) on the host plant × pathogen relationship was studied in order to obtain a better knowledge of the complicated pathogen × host plant × environment interaction.

### Results and discussion

Earlier studies revealed the role of soil-borne pathogens in the development of cold tolerance. In the opinion of some authors, cold tests carried out in the soil demonstrate the efficiency of seed dressing. In order to determine the role played by the *Fusarium* species prevalent in Hungary in chilling tolerance, seed of the complete diallel was inoculated with four species and then germinated at 13.5°C. The chilling tolerance of the lines and hybrids was evaluated after 28 days.

In the control treatment, where only the effect of low temperature was manifested, the dry matter production of the shoots was 50% greater in the hybrids than in the lines. As the result of inoculation the shoot dry matter of the lines decreased more intensively than that of the hybrids, leading to a change in the hybrid:parent ratio. After inoculation with *Fusarium poae* the extent of heterosis was over 200%, four times greater than in the control treatment (Figure 1).

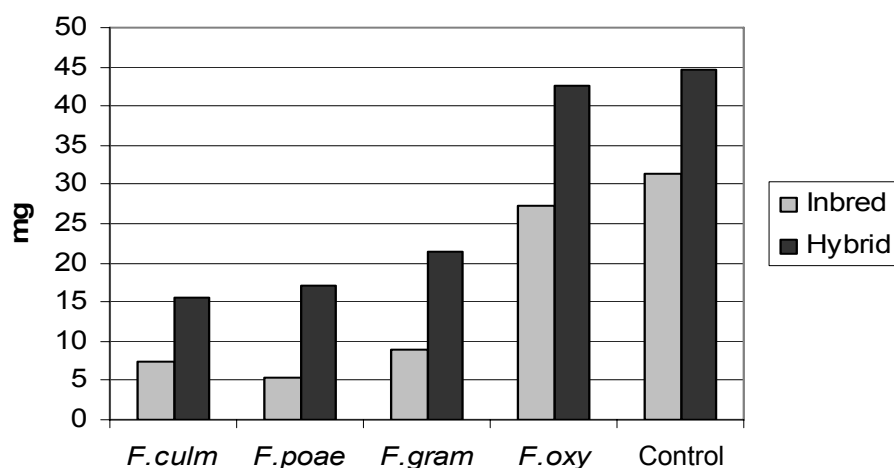


Figure 1. The influence of *Fusarium* infection on individual dry shoot weight.

The results of diallel variance analysis indicated that the ratio of additive to non-additive genetic variance differed in the control and inoculated treatments. While in the control treatment the SCA or non-additive genetic variance was greater than the additive variance, the opposite was true in the inoculated treatments (Table 1).

**Table 1. The diallel analyses of individual dry shoot weight.**

| Source of the variance | MQ |                      |                    |
|------------------------|----|----------------------|--------------------|
|                        | FG | F. oxy               | Control            |
| GCA                    | 6  | 158.6 <sup>***</sup> | 55.5 <sup>*</sup>  |
| SCA                    | 21 | 96.8 <sup>***</sup>  | 82.5 <sup>*</sup>  |
| GRE                    | 6  | 158.1 <sup>***</sup> | 130.0 <sup>*</sup> |
| SRE                    | 15 | 28.8 <sup>NS</sup>   | 26.3 <sup>NS</sup> |
| Error                  | 96 | 28.8                 | 26.3               |

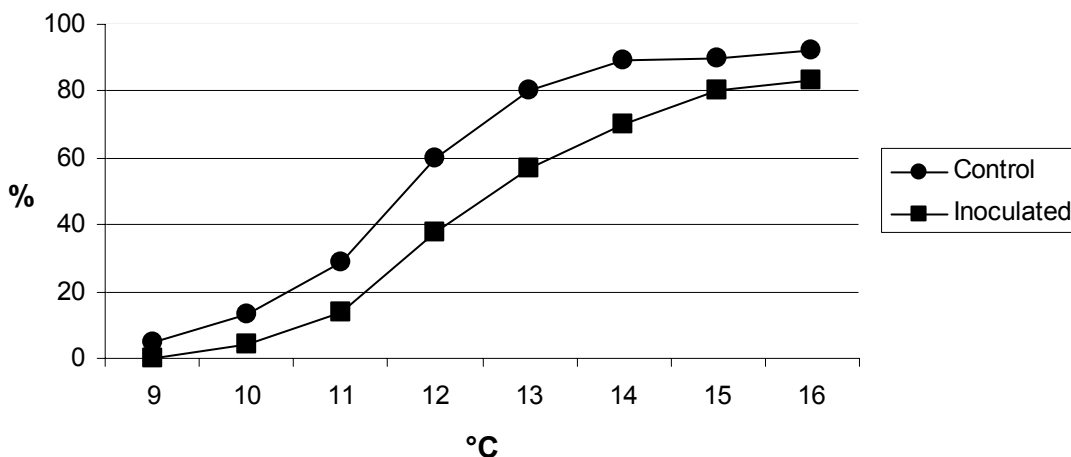
**Table 2. Ranking of cold tolerance (CT) in the inbred lines.**

| Line   | CT at emergence | CT at seedling stage |          |
|--------|-----------------|----------------------|----------|
|        |                 | Extent of growth     | Survival |
| CM174  | 1               | 3                    | 6        |
| HMv307 | 2               | 6                    | 5        |
| A654   | 3               | 1                    | 1        |
| HMv16  | 4               | 2                    | 4        |
| F2     | 5               | 5                    | 2        |
| Mo17   | 6               | 4                    | 3        |

The effect of untested soil-borne pathogens is probably responsible for the contradictory results on the inheritance of chilling tolerance in the literature. In both treatments, however, the general reciprocal effect (GRE) was large and significant, indicating the decisive influence of the female parent in chilling tolerance.

The host plant × pathogen interaction was studied in the following experiment as a function of the environment, in this case the temperature.

The seed of six inbred lines was inoculated with four *Fusarium* species and then sown in sterilized soil. The control seeds were soaked in water. Germination was carried out at ten different temperatures ranging from 9 to 18°C with 1°C intervals in a gradient chamber. As the result of cold there was a considerable deterioration in the emergence percentage even in the control: below 10°C the emergence percentage was only 10%. Inoculation intensified the effect of the cold and reduced the percentage emergence by a further 10–20% (Figure 2).

**Figure 2. The emergence of the inbreds in the range of 9–16°C.**

There was a substantial increase in the emergence time as the result of cold. A temperature difference of 7°C tripled the emergence time. Inoculation did not have any great influence on emergence time. Inoculated seeds only emerged 1–2 days later than the control at below 13°C. The individual shoot dry matter dropped to a tenth as the result of a 7°C difference in temperature, and this was further aggravated by inoculation. Even at 16°C, the mean temperature in May, plants in the inoculated treatments only produced 60% as much dry matter as the control plants.

The degree of tolerance can be obtained by expressing the values of the inoculated treatments as a percentage of the control. The three characters studied gave different values for the level of resistance. The emergence time study indicated 100% resistance almost irrespective of temperature (Figure 3). In the case of emergence percentage and dry matter production, the level of resistance depended on the temperature. The lower the temperature the lower the level of resistance. According to Hooker (1956) germinating seeds are almost completely defenceless against pathogens during the first 1–2 days of germination, after which their defence system develops rapidly. However, what takes 1–2 days under normal conditions takes a lot longer at low temperature, so seeds germinating at 9–10°C remain vulnerable for a longer period, leading to more intense infection. At 16°C, on the other hand, where the emergence percentage is close to 100%, the shoot dry matter production is still only 60% of the control. In other words, even if early infection does not have any great effect on the plant density, it may cause up to 40–50% retardation in seedling development. A 100% yield cannot be expected from such a weakened plant stand.

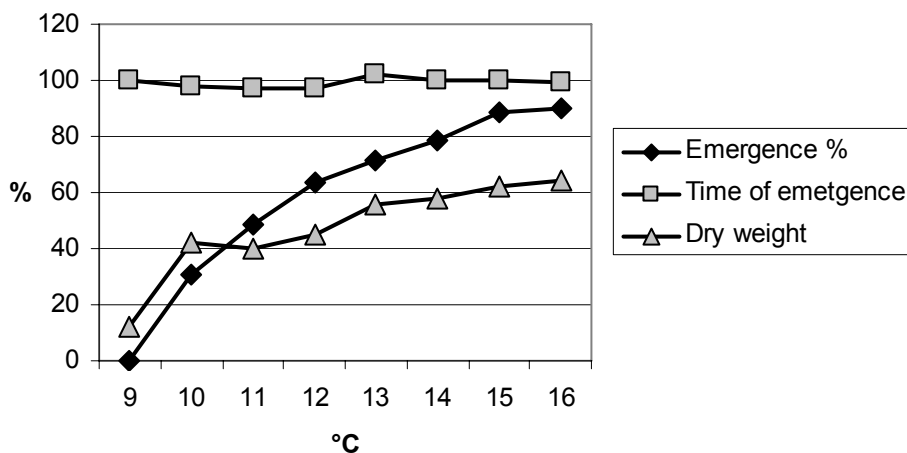


Figure 3. The level of resistance to *Fusarium* spp. in the range of 9–16°C.

The chilling tolerance of six inbred lines at emergence and in the seedling stage was examined over a temperature range of 9–14°C in a gradient chamber. The chilling tolerance of the lines was ranked on the basis of the results. The most chilling-resistant line was found to be CM 174 and the most sensitive Mo 17. Seedling chilling tolerance was judged by germinating the seeds at 15°C and then exposing the seedlings to cold treatment in the two-leaf stage. On the basis of leaf area, leaf number and shoot dry matter the lines were again ranked for chilling tolerance. In this case the most chilling-tolerant line proved to be A 654 and the most sensitive HMv 307. It was concluded that the genetic control of chilling tolerance at emergence was independent of that in the seedling stage, so selection should be carried out separately for the two types of chilling tolerance. Seedling chilling tolerance was also adjudicated in a third way by measuring the percentage shoot dry matter of the plants after 28 days. The dry matter percentage of healthy plants was around 10%. At temperatures



below 10°C the dry matter percentage of some lines (CM 174, HMv 307) was as high as 50%, indicating mortality, while that of other lines (A 654, F 2) was around 20%.

The dry matter percentage thus suggests another way of evaluating chilling tolerance, considering not the extent of growth during the cold period, but the ability to survive the cold period and maintain viability. When evaluated in this way, yet another ranking order was obtained for the chilling tolerance of the lines, with A 654 and F 2 proving the most tolerant and CM 174 the most sensitive. These two types of chilling tolerance have developed as a result of adaptation to different types of climate: under a continental climate there is rarely more than 1 or 2 weeks of cold weather after sowing, so genotypes that can grow intensively even in the cold are more favourable. Under oceanic climatic conditions, on the other hand, the weather warms up more slowly, so longer periods of cold may be experienced, thus favouring genotypes that grow more slowly but retain their viability for a longer period in the cold.

On the basis of the results we can summarize:

- The genetic control of C.T. at emergence and at seedling stage is different, so we have to carry out selection for both
- Two forms of C.T. at seedling stage can be distinguished:
  - intensive growth under short period of cold
  - capability to survive long period of cold
- The genotype of the female parent has a decisive role in CT
- The level of resistance to *Fusarium* spp. plays an important role in the manifestation of CT
- The genetic parameters in the inheritance of CT are modified by the *Fusarium* inoculation
- Breeding can contribute to the improvement of CT, and thus to the increase of maize-growing area in northern countries.

During recent years we have developed some hybrids which could be produced in areas where maize production was not possible before.

## References

- Berzsenyi Z., A.Y. Ragab and D.Q. Lap. 1999. A vetésidő hatásának vizsgálata kukorica (*Zea mays* L.) szemtermésének növekedési dinamikájára Richards-függvényvel. *Növénytermelés* 48:167–187.
- Burris, J.S., O.T. Edje and A.H. Wahab. 1969. Evaluation of various indices of seed and seedling vigor in soybeans. *Proc. Assoc. Off. Seed Anal.* 59:73–81.
- Clark, B.E. 1954. Factors affecting the germination of sweet corn in low temperature laboratory tests. Bulletin No. 769. New York State Agricultural Experiment Station, Geneva, New York.
- Delouche, J.C. and C.C. Baskin. 1973. Accelerated aging techniques for predicting the relative storability of seed lots. *Seed Science and Technology* 1:427–452.
- Hooker, A.L. 1956. Association of resistance to several seedling, root, stalk, and ear diseases in corn. *Phytopathology* 46:379–384.
- Hoppe, P.E. 1955. Cold testing seed corn by the "Rolled Towel Method". Pp. 1–5 in Bulletin 507, Agricultural Experiment Station, Madison.
- Isely, D. 1950. The cold test of corn. *Proceedings of the International Seed Testing Association* 16:299–311.
- Koehler, B. 1957. Pericarp injuries in seed corn: Prevalence in dent corn and relation to seedling blights. III. *Agricultural Experiment Station Bulletin* 617.
- Mathews, S. and W.T. Bradnock. 1968. Relationship between seed exudation and field emergence in peas and French beans. *Horticultural Research* 8:89–93.
- Moore, R.P. 1962. Tetrazolium as a universally acceptable quality test of viable seed. *Proceedings of the International Seed Testing Association* 27:795–805.
- Szirtes J., Barla-Szabó G., Papp D. 1982. Módszer a kukorica vetőmagvak élettani vigorának meghatározására. A kelés előrejelzése különböző stresszviszonyok között. *Vetőmaggyártás*, 9/1: 59–68 [in Hungarian].
- Tatum, L.A. 1942. The effect of genetic constitution and processing method on the ability of maize seed to germinate in cold soil. PhD Thesis, Iowa State College, Ames, Iowa. 80 p.

## **Frost and hail tolerance in quinoa crop and traditional knowledge to handle those adverse factors**

*Alejandro Bonifacio*

### **Introduction**

In the highlands of Bolivia, the native species cultivated are tubers (*Solanum tuberosum* ssp. *andigenum*, tuberous *Oxalis*, *Ollucus tuberosus*, *Thropaelum tuberosum*) and grains (*Chenopodium quinoa*, *Chenopodium pallidicaule* and *Lupinus mutabilis*). Among the grains, quinoa is the most important one.

Quinoa is an annual species cultivated mainly for grain production. The quinoa grain is used in ways similar to rice or wheat. For human consumption, quinoa is used for different dishes and cookies; it can be boiled, milled, flaked and toasted.

The quinoa crop is cultivated in the high, dry and cold areas of Bolivia and Peru. The main abiotic stressors for quinoa are drought, frost, hail and soil salinity. Frost occurs during 200–220 days per year and the hail occurs almost at random during the rainy season. Frost and hail cause reduction or yield loss, but their effects have not been well studied. Farmers from the highlands know the risks of frost and hail for quinoa crop; they also know some indicators for frost and hail occurrence. Knowing the high risk from these natural phenomena, farmers have developed some strategies to prevent their effects. However, these practices have not been documented.

A case study was carried out with the following objectives:

- To describe the injuries or lesions caused by frost and hail in quinoa
- To evaluate the effect of frost and hail in quinoa varieties and ecotypes
- To gather traditional knowledge to predict and to reduce the effects of frost and hail.

### **Materials and methods**

The case study was carried out at the Choquenaira Experiment Station and Jalsuri rural community located in Viacha municipality, Ingavi province of La Paz department, Bolivia.

The genetic material included in the study was improved varieties, breeding lines and germplasm accessions.

### **Frost**

The effect of frost was studied in field conditions under natural occurrence of frost during the quinoa growing period. The low temperature was recorded by a maximum-minimum thermometer placed in the research plot.

Evaluation of the effect of frost was carried out in two instances. The first was between 2 and 3 hours after frost occurrence and the second one during the plant recovery period. The evaluation was conducted using a scale of 5, grading 1 for tolerance and 5 for susceptibility.

The lesions or injuries caused by the frost have been divided into primary lesions and secondary effects. Primary lesions refer to direct injuries to the plant as a consequence of low temperatures, from  $-5.5$  to  $-6.0^{\circ}\text{C}$ . Secondary effects refer to the physical, morphological and pathological consequences in the affected plants during the plant recovery process.

### **Hail**

Quinoa lines were evaluated according to the hail injuries. As in the case of frost, hail injuries were evaluated on a 1 to 5 scale, and descriptions of the lesions and physical injuries in different parts of the plant were recorded.

The direct lesions caused by hail were described immediately after hail occurrence. The secondary effects of the hail were evaluated during the process of plant recovery based on the plant architecture or plant 'type' compared with non-affected plants. The consequences of

hail injury are described as pathogenic infections, physical lesions, and other adverse effects in the plant.

### Local knowledge on frost and hail

Traditional knowledge to predict the occurrence of frost and hail was gathered from oral information and testimonies of the producers described in their native language (Aymara).

The traditional information includes indicators for frost and hail occurrence as well as the traditional practices of 'fighting' against the frost and hail phenomena. This information was recorded one or two days after the occurrence of the frost and hail.

## Results and discussion

### Frost

In the highlands of Bolivia, the occurrence of frost 200–220 days a year results in a very short benign period to grow crops (November to March). Even in this benign period there is a high frost risk for crops.

The adverse effects of the frost in the quinoa consist of a series of damages and lesions that lead to yield reduction; in severe cases it can cause the total loss of yield.

The direct effects of the frost on the plant are death of apical leaves and stem epidermis, total or partial injury of the panicle, flower abortion, etc. The secondary effects of the frost are tortuous growth of the stem, branching, defoliation, cracks in the stem and infection with pathogens (rot).

The genetic material included in the preliminary evaluation shows different degrees of tolerance to frost (Table 1). The variety Chucapaca was the most tolerant to frost and the accessions and lines 0694, 1560, ECU 420 and EDK 95 were the most susceptible. This indicates some variation among the genetic material for frost tolerance. Plant material graded as susceptible comes from valleys where the frost almost never occurs, thus it reflects the evolutionary aspects implied in frost tolerance.

**Table 1. Main primary and secondary effects of the frost in varieties and quinoa lines (–5.5 to –6.0°C).**

| Variety   | Primary effects   | Secondary effects   | Range | Tolerance <sup>†</sup> |
|-----------|---|---|-------|------------------------|
| 0694      | Death of apical leaves and epidermis of the stem, panicle injury, floral abortion | Tortuous stem, plant lodging, cracks or fissures in the stem            | 4     | SS                     |
| Chucapaca | Frozen leaf borders, curved stem  | Partial defoliation   | 1     | T                      |
| M–389     | Frozen apical leaf borders, curved stem   | Small cracks in the stem, partial defoliation                           | 3     | S                      |
| L–P       | Frozen leaves, death of the upper third of the plant                              | Large cracks in the stem, defoliation, branching, plant lodging         | 3     | S                      |
| 1560      | Frozen leaves, death of the upper third of the plant                              | Average sized cracks in the stem, defoliation, branching, plant lodging | 3     | S                      |
| ECU 402   | Frozen leaves, death of the upper third of the plant                              | Large cracks in the stem, defoliation, branching, lodging               | 4     | SS                     |
| EDK-95    | Death of almost all the plant   | Plant lodging, plant death  | 5     | SS                     |

<sup>†</sup> S = Susceptible, T = Tolerant, SS very susceptible, MT = fairly tolerant.

### Hail

The main damages caused by hail are total or partial breakage of leaves, detachment of leaves or groups of flowers, necrosis of flower and floral abortion. If the plant is not severely affected (tolerant), it can recover; however some adverse consequences show up during plant growth. The main injuries are located in the plant stem, leaves and panicle, especially on the side facing the direction of the hail particle where it hits the plant.

The direct effects of the hail are large numbers of point lesions in the stem, breakage of leaves and leaf petiole, floral abortion. The secondary effects are dry rot in the stem, lack of grain filling, heterogeneity of the grain and others.

The genetic material included in the case study shows a range of hail tolerance, allowing grouping of the material as very susceptible, susceptible, fairly tolerant and tolerant. The line L-320 and the variety Kankolla are tolerant to hail. In contrast, ECU 402 and NL-6 were the most susceptible ones (Table 2).

Another direct effect of the hail on the quinoa is grain shattering, especially when the plant is mature and dry.

The tolerance to hail seems to be associated with a series of morphological and anatomical plant characteristics. Plants that have small leaves, short petioles, small insertion angle of the petiole, and flexible stem are tolerant to hail. So far, breeding for hail tolerance has not been included in the plant breeding objectives, but these results suggest working for this trait.

**Table 2. Main direct effects and sequels of the hail in varieties and breeding lines.**

| Variety   | Direct injuries  | Secondary effects  | Range | Tolerance <sup>†</sup> |
|-----------|--|--|-------|------------------------|
| Sayaña    | Lesions in the stem, breakage of leaves, partial breakage of panicle | Large number of rotten points in the stem, plant lodging   | 3     | S                      |
| Intinaira | Slight lesions in the stem, leaf breakage                            | Small number of rotten points in the stem  | 2     | MT                     |
| Chucapaca | Lesions in the stem, breakage of leaves, partial breakage of panicle | Small number of rotten points in the stem  | 3     | S                      |
| Line 320  | Small point lesions in the stem                                      | Lesion with no rotten points in the stem   | 1-2   | T                      |
| L-P       | Leaf destruction, defoliation, large number of point lesions in stem | Severe lesions in the stem, large number of rotten points in the stem, stem breakage, plant lodging  | 4     | S                      |
| Kankolla  | Small lesion in the stem, point lesion in stem                       | Lesion with no rotten points in the stem   | 1-2   | T                      |
| ECU 402   | Leaf destruction, defoliation, large number of point lesions in stem | Severe lesions in the stem, large number of rotten points in the stem, stem breakage, plant lodging  | 4-5   | SS                     |
| NL-6      | Severe defoliation, leaf destruction, floral abortion                | Severe lesions in the plant, large number of rotten points in the stem, stem breakage, plant lodging | 5     | SS                     |

<sup>†</sup> S = Susceptible, T = Tolerant, SS very susceptible, MT = fairly tolerant.

### Local knowledge on frozen and their effects

According to the testimony of farmers, the risk of frost in the highland varies from year to year and from site to site. The indicators to predict frost and hail are associated with the weather regime, among them the distribution of clouds, wind direction, intensity of solar radiation and high temperature.

Lack of clouds in the sky, low-speed western winds (Atacama Desert) or northern winds (Snow Mountains) are key indicators for frost occurrence in the Bolivian highland.

Key indicators to predict hail occurrence are high diurnal temperature, dark and localized clouds, and lack of wind. The prediction can be made 2–3 hours in advance.

The farmers' knowledge of the climatic indicators allows them to identify areas where frost and hail frequently occur or both phenomena occur at the same time. According to the testimony of the producers, the areas under frost and hail risk are well known. Using this knowledge, they assembled an imaginary map associated with cropping systems. The areas

with high risk of frost are the flat and low plains where the water usually concentrates, whereas the areas with a low risk of frost are the hillsides.

The strategies to handle the risk derived from frost are based on microecosystems, planting dates and varietal characteristics: planting in hillsides where the frost rarely occurs, broader planting dates, late or early maturing varieties according to planting dates. Another practice is to make fires around the planting sites during cold nights.

Regarding hail, farmers know the areas where hail more frequently occurs. These areas are known as 'roads of the hail'. The hail dispersion is localized and it is variable in acreage, covering from dozens up to hundred hectares.

The practices to prevent, or 'to drive away' or to redirect the trajectory of the hail consist of the generation of smoke and the launching of firecrackers. For this and other inherent purposes, each community designates one or two people that are denominated *yapu camani*. In the face of the imminent risk of hail, those *yapu camani* take action to make fire and smoke to alert the neighbours. In response to this alert, farmers and shepherds also make smoke to deflect the trajectory of the hail or to reduce its intensity. This strategy against hail reflects the degree of risk that this phenomenon represents for crops.

### **Conclusions**

The frost causes diverse types of injury in the quinoa plant. These injuries can be described as primary lesions (caused by freezing temperature) and secondary effects of the frost that become evident during the recovery process in affected plants.

The hail injuries to the quinoa plant are diverse and their consequences depend on the phenological phase of the plant and the characteristics of the varieties. Those injuries caused by the hail are described as direct lesions and indirect effects or sequels during the recovery of the plant.

The hail causes defoliation, fractures of the stem, large number of point lesions in the stem and is mostly concentrated in the side where the hail particles hit.

The lesions caused by hail constitute infection points for bacteria that produce the dry rot in the lower part of the stem; in severe attacks it causes plant lodging.

The genetic material shows variation to frost and hail tolerance, which is an indicator for undertaking research for quinoa improvement for this trait.

The traditional knowledge allows farmers to predict the occurrence of frost and hail with some degree of probability and in advance.

The farmers have developed practices of prevention of the hail that involves cropping systems, varietal preference, planting dates, and nominating people (*yapu camani*) to take care of the crops.

## Participatory plant breeding for enhancing the use of local crop genetic diversity to manage abiotic stresses

*Sanjaya Gyawali and Bhuwon R. Sthapit*

### **Introduction**

Rice (*Oryza sativa* L.) is the most important food crop of Nepalese agriculture. In Nepal, rice is cultivated on 1.4 million ha where more than 66% of rice is rain-fed, with attendant drought and flooding stresses each year (Central Bureau of Nepal 2004). It is reported that 9% of rice area is cultivated under extreme drought conditions in unbunded rice field—called the *Ghaiya* ecosystem. Similarly 3% of rice area is under cold tropical regions between 1500 and 3050 m, which is the highest altitude in the world for rice cultivation.

Low productivity of rice has occurred because of high abiotic and biotic stresses as well as narrow varietal options to cope with these stresses. Abiotic stresses such as drought, flooding and chilling injuries are common in Nepal, and these abiotic stresses predispose the rice varieties to biotic stresses such as blast, Bacterial leaf blight and bacterial sheath brown rot diseases, resulting in poor productivity. Sthapit and Witcombe (1998) studied the inheritance of chilling tolerance in Nepalese landraces *Chhomrong* and *Raksali* in high-altitude rices in Kaski. Sthapit et al. (1995) reported the genetic mechanism of Sheath Brown Rot disease associated with chilling injuries in Nepal.

Farmers have narrow varietal choices offered by conventional breeding programmes to manage these stresses on-farm. The introduction of stress-tolerant rice germplasm, from either IRRI or the National Rice Research Program, has resulted in fewer successes in abiotic environments than in favourable and high-potential production systems in Nepal (Sthapit et al. 1996; Joshi et al. 2002). Rana (2004) reported that farmers manage stresses under *Uchha* (rain-fed, banded rice field, drought) and *Nichha* (rain-fed, waterlogged and flooding conditions with poorly drained rice fields) by deploying the 'best fit' from the available local landraces. In this context, participatory plant breeding has been used as a strategy to maximize use of local landraces by using one of the parents in a crossing programme and integrating the farmers' local knowledge of target populations traits (TPTs) (traits tolerant to stresses) and target population of environments (TPEs) (selection under stress conditions on-farm) (Witcombe et al. 2005). Therefore, the PPB program of LI-BIRD in collaboration with NARC, IPGRI, CAZS and farming community has focused on the breeding of rice varieties suitable under stress, especially drought and poor fertility conditions on-farm.

In this regard, LI-BIRD has monitored the spread of *Machhapuchhre 3* (the first released PPB bred variety in Nepal) in the western hills of Nepal (Sthapit et al. 1996; Joshi et al. 1997) and assessed the impact of varietal spread in high-altitude rices (Joshi et al. 2001). Highly drought-tolerant rice varieties such as *Barkhe 1027*, *Sugandha 1*, *Judi 572* and *Judi 582* have been bred by the PPB programme of LI-BIRD and have been spread through farmers seed networks in Nepal and Bangladesh (Gyawali et al. 2002; Witcombe et al. 2005). In IPGRI's *in situ* conservation on-farm project, the rice varieties adapted to drought and poor fertility, such as *Mansara* and *Aanga*, have been used in a PPB programme to add value to these valuable stress-tolerant varieties on-farm with farmers' preferred traits. This paper summarizes the PPB approaches employed to make these locally adapted landraces with stress tolerance and other relevant traits more competitive with the available options in the market. The deployment of PPB-bred varieties on-farm and its impact on varietal spread is discussed.

## Materials and methods

### Case study 1: Understanding management of stress tolerance through deploying rice landraces

In baseline studies of *in situ* conservation of agrobiodiversity in Nepal, the four-cell analysis was used to identify and characterize the varietal diversity on-farm. Based on this analysis the parents for PPB in the *in situ* project were selected (Sthapit et al. 2001). We employed Social Analysis Tool–Construct Analysis (Chevalier 2005) to understand the farmers' management of stress tolerance in rice in Kaski. The baseline information on varietal characteristics of rice landraces was verified in a focus group discussion to understand farmers' management practices for abiotic stresses using locally available genetic diversity. We invited two male and two female key informants and nodal farmers who had knowledge of local landraces from Begnas village. Seed samples of landraces were displayed for farmers to validate variety names with the seed morphotypes and their individual characteristics on stress tolerance were verified against the baseline information. Farmers were facilitated in developing a list of matching characteristics of stress tolerance in rice landraces (Tables 1, 2).

**Table 1. Rice landraces used in the assessment of stress tolerance in Begnas, Kaski, 2005.**

| Rice landraces |  |
|----------------|--|
| Darmali        | Thulo Gurdi  |
| Aanga          | Jethobudho   |
| Mansara        | Anadi Seto   |
| Pakhe Jhinuwa  | Anadi Rato   |
| Naltumme       | Mansuli  |
| Kathe Gurdi    | Panhele  |
| Rate           | F <sub>6</sub> advance lines of Mansara/Khumal 4 (PPB bred materials)  |
| Bayerni        | F <sub>6</sub> advance lines of Biramphool/Himali (PPB bred materials) |
| Dhabe Jarneli  |  |

Source: Focus Group Discussion with key informants in Kaski, 2004.

**Table 2. Matching characters considered important by farmers to manage abiotic stress on-farm through deployment of rice landraces.**

| List of matching traits to stress tolerance |                     |
|---|---------------------|
| Rain-fed but not drought                    | Drought             |
| Irrigated                                   | Partially irrigated |
| High fertility                              | Poor fertility      |
| Productive land category                    | Poor land category  |
| Cold water source                           | Hot water source    |
| Sunny rice field                            | Shaded rice field   |
| Flat land                                   | Terrace rice field  |
| Late maturity                               | Early maturity      |
| High grain yield                            | Low grain yield     |
| Good eating quality                         | Poor eating quality |

Source: Focus Group Discussion with key informants in Kaski, 2004.

The landraces were scored on a 1 to 5 scale based on their ability to tolerate the stresses. For each farmer variety the landrace tolerance to stresses was discussed in a group and scored for all matching traits, ranging from low to high. Farmers were requested to score the PPB products, especially the selected progenies of *Mansara/Khumal 4* and *Biramphool/Himali*. The scores were compiled and entered into the construct analysis of SAS software (Rep IV Personal Version 1.0). Construct analysis of SAS used multivariate (Cluster and Principle Component) analysis to draw dendrogram and biplot figures (Gaines and Shaw 2004).

**Case study 2: Rain-fed, low-input conditions**

Participatory plant breeding (PPB) using the abiotic stress tolerant local landrace *Mansara* was explained by Gyawali et al. (2004a). The four-cell analysis used to analyze the positive and negative traits in local landraces and complementary parents was selected by the breeders (Table 3). The crossing was made on-station and  $F_1$  in advanced on-station trials by the plant breeder. The  $F_2$  through to  $F_{6-7}$  were evaluated adopting a large population size strategy to recover rare segregants on-farm and progenies were selected on-farm together with farmers (Table 4).

**Table 3. Parents and their valued and negative traits analyzed during parent selection in PPB programme of *in situ* conservation project in Begnas, Nepal.**

| Local parent | Valued traits   | Negative traits to be improved in landrace   | Improved parents |
|--------------|---|--|------------------|
| Anga         | <ul style="list-style-type: none"> <li>• Drought tolerant, adapted to poor soil fertility</li> <li>• Has medicinal value</li> <li>• Water-soaked rice is perceived as coolant in heat stress; straw has similar effect for animals</li> </ul> | <ul style="list-style-type: none"> <li>• Low yield</li> <li>• Short panicles</li> <li>• Low straw yield</li> </ul> | NR 10291         |
| Mansara      | <ul style="list-style-type: none"> <li>• Tolerant to drought and poor soil fertility (marginal) conditions; only rice grown in very poor soil</li> </ul>  | <ul style="list-style-type: none"> <li>• Poor eating qualities</li> <li>• Low yield</li> </ul>                     | Khupal 4         |

Adapted from Gyawali et al. 2004a.

**Table 4. Crosses and population sizes in  $F_3$  generation in PPB programme in Begnas, Nepal.**

| Cross            | Kholakochheu site      |              | Begnas site            |              | Total popln. |
|------------------|------------------------|--------------|------------------------|--------------|--------------|
|                  | Area (m <sup>2</sup> ) | Plant popln. | Area (m <sup>2</sup> ) | Plant popln. |              |
| Anga/NR 10291    | —                      | —            | 250                    | 8333         | 8333         |
| Mansara/Khupal 4 | 150                    | 5000         | 700                    | 23333        | 28333        |

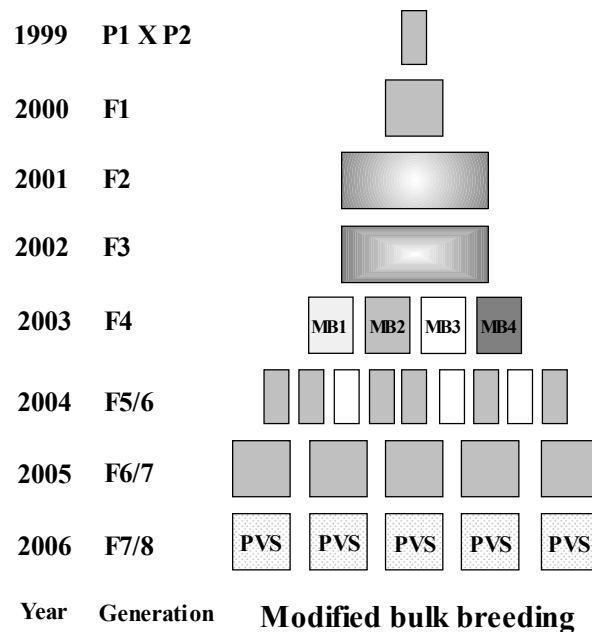
Adapted from Gyawali et al. 2004a.

**Modified bulk breeding methods**

Modified bulk breeding and pure line selection from bulk breeding method (Figure 1) were used in the present study. In the modified bulk breeding method, the bulk derived from cross *Mansara* /*Khupal 4* was divided into three groups based on the grain type and colour at  $F_3$  generation. For example, the cross *Mansara* /*Khupal 4* was modified into *Mansara* type (dark red pigmented colour), intermediate type (faded red colour) and *Khupal 4* type (Straw colour). The target population of traits (TPTs) was identified in focus group discussion during a travelling seminar for developing modified bulk. Farmers identified the best plants from the bulks based on the grain type and colour during modification of the bulks.

Farmers were requested to select the desired plant types from a large population. In 2002, Ms Ganga Pandey selected the *Mansara* types from the modified bulk and grew it in her own plot in 2003. The neighbours observed that the bulk was superior to local *Mansara* in Ganga's plots. So, Mr Manhari Kanal, Mr Rudra Nath Adhikari, Mr Bharat Raj Tiwari and Lila Nath Dhakal selected the desired plant types from the modified bulk from breeding block in 2003. A team of farmers, field technicians, PPB thematic members and researchers jointly selected the best hills to purify the family rows in 2004.





**Figure 1. Modified bulk breeding (adapted from Gyawali et al. 2002) for PPB in the *in situ* conservation project in Kaski, Nepal.**

### Case study 3: Breeding for chilling tolerance

The Nepal Agriculture Council (NARC) initiated breeding for cold tolerance and blast resistance through selection of local landraces named *Jumli Marshy* in Jumla but there was less variation within this population. Many new exotic germplasms were tested but these were found less adaptive; in most cases not flowering in Jumla conditions. However, Sthapit and Witcombe (1998) studied rigorously the genetic mechanism of chilling tolerance of Nepalese landrace including *Chhomrong Dhan* and *Raksali* from Kaski. They estimated the heritability of chilling tolerance which ranged from 0.7 to 0.9. Sthapit et al. (1995) also reported the genetic mechanism of Bacterial Sheath Brown rot (ShBR) associated with cold injuries in high-altitude rices in Nepal. Very high heritability and high variances in genetic mechanism of resistance to this disease have led to development of resistant cultivars suitable for high-altitude rices. During the 1990s this highly heritable chilling tolerance and resistance to ShBR in *Chhomrong Dhan* was effectively used in breeding M 3 and M 9 through PPB approaches. Recently scientists from ABD of NARC have initiated breeding for cold tolerance and blast resistance. Bajracharya (2004) compared genetic diversity of rice landraces from terai (Bara), middle hills (Kaski) and Jumla (high hills). The participatory plant breeding (PPB) on high-altitude rice was initiated by scientists in collaboration with farmers by LARC in *Chhomrong* and *Ghandruk* (>1500 masl) villages of Kaski, Nepal (Sthapit et al. 1996). In this PPB programme, Fuji 102 was crossed with *Chhomrong Dhan* (cold-tolerant and red-grained paddy but susceptible to bacterial sheath brown rot) and F<sub>1</sub> and F<sub>2</sub> advanced in on-station trials. The seeds of F<sub>3</sub> onward were screened and selected under target environments and farmers' management conditions. Initially 12 farmers participated in the PPB programme of M 3 and M 9 derived 10 bulks. These bulks were further screened and evaluated by farmers to derive M 3 and M 9 bulks. In 1996, M 3 was officially released by the National Seed Board of Nepal as a cold-tolerant rice variety for the in high hills of Nepal.

LI-BIRD initiated monitoring of high-altitude rices of M 3 and M 9 from 1997 to 1999 (Joshi et al. 2001) and continued study on its spread in 2003 and 2004 using household-level surveys and direct observation in the field. In 2003 and 2004, the same farmers who had grown M 3 and M 9 in 1999 were revisited and interviewed. The survey revealed that these two varieties have spread in the new villages of neighbouring districts through farmer-to-

farmer seed networks, NGO and GO initiatives. Therefore, new farmers who received seed of M 3 and M 9 during 2000 to 2002 were traced, visited and interviewed as well. The area and number of farmers growing these varieties were determined by interviews. Farmers' perception on the performance of new varieties and reasons for adoption or rejection were recorded.

## **Results**

### **Case study 1: Understanding management of stress tolerance through deploying rice landraces**

The dendrogram and biplot PCA for landraces and their relevant abiotic stress tolerant traits in Begnas conditions are presented in Figures 2 and 3.

We found that unfertile land, drought, partial irrigation and poor land category are highly correlated. Similarly, a shaded rice field is closely associated with poor grain quality. The construct analysis revealed that *Darmali* and *Aanga* can tolerate extreme drought and poor fertility conditions but we noticed their grain yield was scored as very poor. It is considered that these two varieties are grown in those fields where no other rice landraces/varieties survive. However, *Mansara* and *Pakhe Jhinuwa* together with *Darmali* and *Aanga* developed Cluster I, which is highly drought-tolerant and adapted to poor soil fertility conditions. In this cluster, *Pakhe Jhinuwa* is considered to be superior in terms of eating quality whereas *Aanga* has medicinal values but two other landraces have poor eating qualities (Figures 2 and 3).

Cluster II comprises *Naltumme*, *Kathe Gurdi* and *Rate* which are considered less tolerant to abiotic stress than Cluster I. However, *Naltumme* has unique adaptation to shaded micro-niches (Figures 2 and 3). Farmers gave the highest score to *Naltumme* for its higher shade tolerance and reported that its performance in terms of grain yield is higher in shaded conditions than in sunny (open) rice fields but its grain quality under shaded conditions is extremely poor.

The third cluster includes *Dhabe Jarneli*, *Thulo Gurdi* and *Bayerni* which require more favourable conditions (Figures 2 and 3). These landraces require relatively higher fertility and well-irrigated rice fields to perform better. However, this category of rice landrace gives satisfactory yield under relatively poor soil fertility as well as drought conditions but farmers scored the cluster medium in terms of abiotic stress tolerance. Cluster IV comprises *Anadi Seto*, *Anadi Rato* and *Jethobudho*. This category of rice landraces furnished the cluster having landraces with aromatic and superior grain quality, late maturity and higher grain yield. The unique trait of this cluster is cold water tolerance which enhances the grain quality. We found *Panhele* and *Mansuli* to be outside of any cluster because these varieties could not tolerate drought and require highly fertile flat land. *Panhele* is a rice landrace that is native to Kaski valley whereas *Mansuli* has been one of the most popular rice varieties in Nepal for the last three decades.

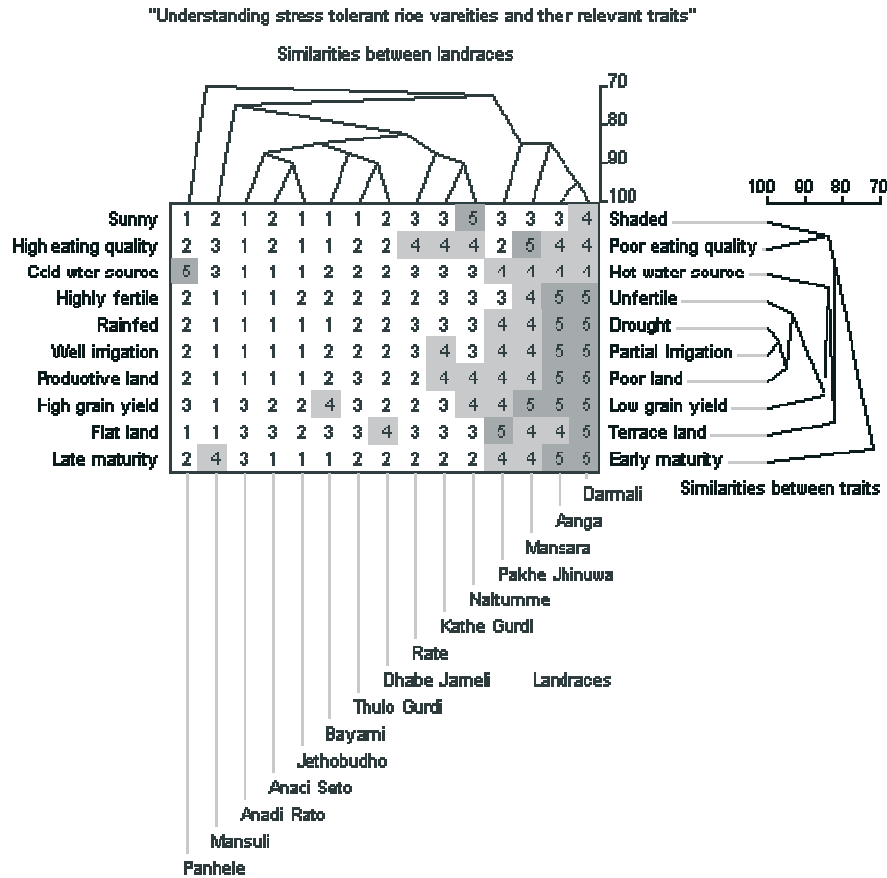


Figure 2. Dendrogram of rice landraces and their relevant stress-tolerant traits analyzed for Begnas, Kaski 2005.

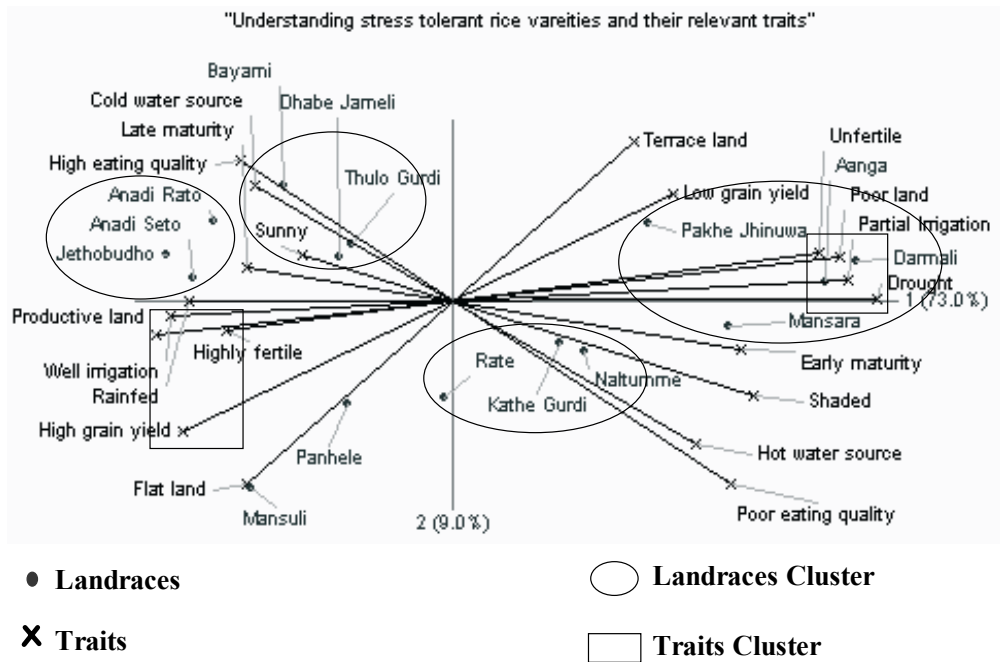


Figure 3. Principal component analysis of rice landraces and their stress-tolerant traits in Begnas, Nepal, 2005.

### Case study 2: Rain-fed, low-input conditions (Collaborative breeding by farmers for Mansara/Khumal 4)

In 2002, the seed of segregating materials was received back from farmers to maintain the larger plot size and selected for rare segregants from the population of modified bulks. The populations were grown in large rented block (>25 000 plants maintained) and farmers were invited to select within the segregating bulks. Mr Bharat Raj Tiwari selected some of the attractive panicles from the rented block of *Mansara/Khumal 4* cross and advanced the materials in his farm. The neighbours of Mr Tiwari noticed rice bulk looking similar to *Mansara* but promising for higher grain yield (larger panicles and higher grain per panicle) in his field. The neighbours regularly observed his field and asked him to provide seed for the 2003 planting. Farmers were very much attracted towards this cross. In 2003, five more farmers (*viz.* Ms Ganga Adhikari, Mr Manahari Kandel, Mr Rudranath Adhikari, Mr Lilanath Dhakal and Ms Tiwari), selected panicles from this cross in 2003. The population of this cross was considerably high in  $F_2$  and succeeding generations. This perhaps led to the segregation of more rare segregants where researchers and farmers selected those genotypes from the population. Similarly, Mr Budhi Sagar Tiwari from Ralmare (a farmer from outside the PPB village area) received 4 kg seed (remnant seed) from the rented block of *Mansara/Khumal 4* cross and tested it in his farm in 2004. Farmer-selected bulks were compared with local *Mansara* and it was found that the selected bulks were more drought-tolerant and have higher grain yield with improved eating quality.

In 2004, a travelling seminar was organized to monitor and select the best progenies before crop maturity. During the travelling seminar, farmers and scientist jointly selected 23 populations out of 99 from previous years. Immediate after this, farmers of the PPB group independently organized a travelling seminar for non-participating farmers only into the PPB blocks and farmers selected the 7 best populations to advance in their own fields.

### Case study 3: Abiotic stress (chilling injuries) and associated biotic stress – PPB: an option for breeding farmer-preferred varieties and its impact on varietal diversity on-farm

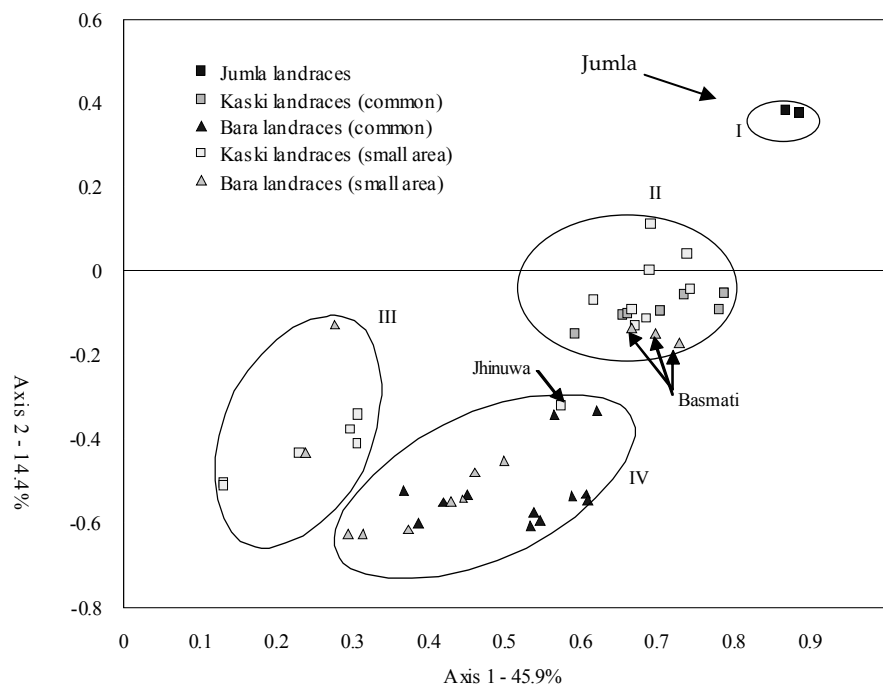
Bajracharya (2004) found that the landraces from high hills of Jumla seemed diverse based on agromorphological characters such as grain type, grain colour and panicle characters but she reported that landraces from this highly chilling-prone district had an extremely narrow genetic base (as determined by SSR marker analysis) compared with other agroecological zones (Table 5). She further concluded that this narrow genetic base might be due to the natural selection of high chilling and or cold stress over many years (Figure 4). Compared with Clusters II, III and IV, Cluster I has an extremely narrow genetic base, indicating Bara and Kaski had higher genetic diversity.

**Table 5. Genetic diversity parameters measured for rice varieties in Bara, Kaski and Jumla using SSR markers in 2004.**

| Diversity parameters                            | Jumla <sup>†</sup> | Kaski | Bara |
|---|--------------------|-------|------|
| Total number of primers                         | 39                 | 38    | 38   |
| Total number of polymorphic primers detected    | 1                  | 34    | 33   |
| Total number of alleles amplified               | 40                 | 91    | 95   |
| Average number of alleles per primer (A)        | 1.0                | 2.4   | 2.5  |
| Percentage of polymorphic bands (PPA)           | 2.5                | 95.6  | 94.7 |
| Average number of alleles per poly. Primer (Ap) | 1.0                | 2.6   | 2.7  |
| Average genetic dissimilarity                   | 0.12               | 0.45  | 0.45 |
| Average gene diversity (PIC)                    | 0.05               | 0.37  | 0.40 |

Adapted from Bajracharya (2004). <sup>†</sup> Jumla=Cold injuries, Kaski=Chilling injuries, Bara=Drought stress.

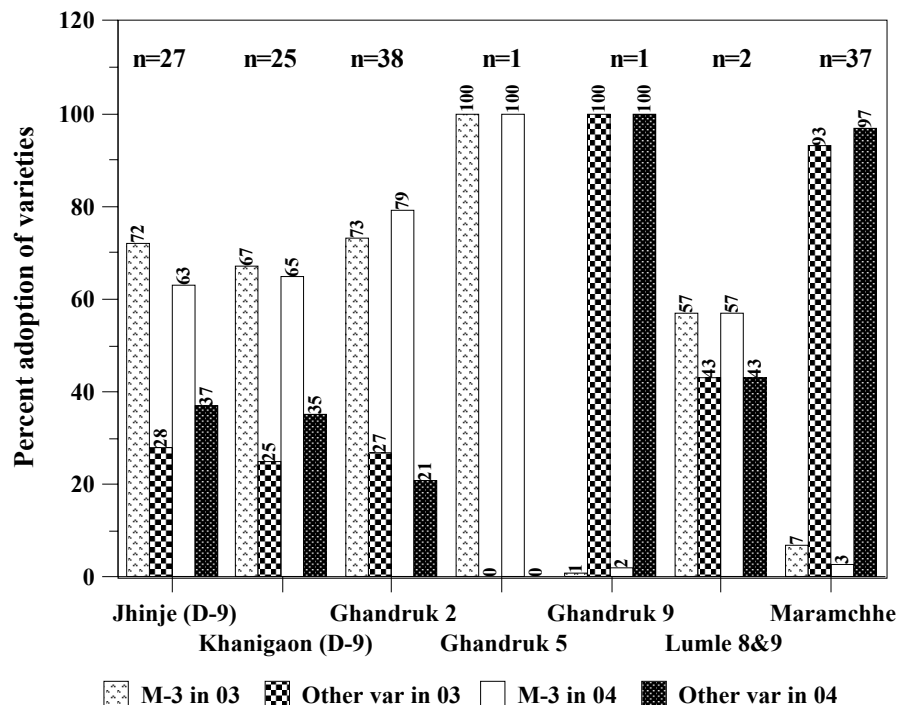
Bajracharya (2004) reported that cold stress could affect the genetic diversity of landraces and predispose these landraces to other stresses such as disease and insect pests. *Jumli Marshi* has higher cold tolerance but is highly susceptible to leaf and neck blast. The breeding for blast resistance retaining higher cold tolerance of *Jumli Marshi* has promised little success in the past but Sthapit et al. (1996) demonstrated that PPB was successfully employed in developing cold-tolerant varieties through PPB in high-altitude rices in Nepal. A successful story of chilling tolerance and bacterial sheath brown rot resistance breeding came from Lumle Agriculture Research Center (LARC) during the mid- to late 1990s. During that period, the chilling tolerance and ShBR resistance (0.7–0.9) were introgressed into M 3 and M 9 varieties by a team of scientist in LARC. Very high heritability of chilling tolerance and ShBR resistance was reported by Sthapit and Witcombe (1998) and Sthapit et al. (1995) in *Chhomrong Dhan* and *Raksali*, which are valuable genetic resources used and maintained by farmers in managing these abiotic and associated biotic resources as well as future breeding programmes.



**Figure 4. Cold stress affecting rice landrace diversity in Jumla (high-altitude rice) in Nepal measured using SSR markers in 2004 (adapted from Bajracharya 2004).**

Participatory variety selection of PPB bred varieties has greatly enhanced farmers' access to these varieties on-farm. Joshi et al. (2001) monitored the impact of PPB-bred varieties on livelihood and varietal diversity on-farm. They reported that the adoption rate on-farm of M 3 and M 9 is as high as 60% depending on villages interviewed. However, the adoption of PPB-bred varieties was a dynamic process where farmers evaluated these varieties, some continuing to grow them and others dropping them. In 1996, farmers of Kande grew M 3 in more than 45% of the village rice area, but it was as low as 5% in Marangche. In the same year, farmers of Kande grew M 9 in less than 5% area but its adoption was the highest (>60%) in Marangche village. In 2003 and 2004, the adoption of M 3 was reported as 3.3% and 2.4%, respectively, in those villages, whereas M 9 was 18% and 15%, respectively (Figure 5). In 2004, we noticed that a new variety named *Lumle-2*, an output of another cross *IR64/Chhomrong Dhan* from a PPB programme, steadily increased its area (14% and 24% area in 2003 and 2004, respectively) in Marangche. Farmers reported the eating quality of *Lumle-2*

as superior to M 3 and M 9 whereas the grain yield as well as cold tolerance was comparable. The field survey of *Chhomrong* and *Ghandruk* could not be completed due to security reason, however; the overall adoption of PPB bred varieties was still higher 48% and 46% in 2003 and 2004 respectively.



**Figure 5. Adoption of cold-tolerant rice varieties M 3 and M 9 in Kaski district during 2003 and 2004.**

### Discussion

Understanding the existing genetic pool at community level and its deployment in managing abiotic stresses is extremely helpful. Initially the four-cell analysis—which is based on number of households growing varieties and area occupied—was used to understand the existing diversity of rice landraces at community level. However, the landraces, their traits relevant to stress tolerance, and landrace deployment as a strategy of stress management have not been explored in depth. The SAS software that we used was very helpful in understanding the dynamics of landrace management at community level. Farmers of Begnas village of Kaski have unique landrace diversity specifically adapted to particular stress. The landraces of Cluster I and II have high tolerance for drought and poor fertility and are an important contribution to the livelihood of poor farmers who cannot afford high-input rice cultivation. Similarly, farmers also have choices for favourable production environments where landraces compete with improved varieties. Farmers maintain those landraces with higher grain quality to compete with higher grain yield of modern varieties. *Jethobudho*, *Panhele*, *Bayerni* and *Anadi* are some example of landraces that fetch higher prices at market to compete with the high grain yield of *Mansuli*, a modern variety. Furthermore, the high-quality landraces have cold water tolerances which in fact improve the grain quality of these landraces (Gyawali et al. 2004b).

Farmers deploy landraces based on stresses encountered in particular rice fields. *Naltumme*, a shade-loving rice landrace, performs extremely well under shaded rice fields with hot water sources. Bajracharya (2004) reported that the landraces of Begnas are highly

diverse as determined by agromorphological as well as molecular markers. She reported that these landraces have unique alleles conferring specific adaptation and traits but the landrace diversity in Jumla was reported extremely narrow due to the higher cold stress there, resulting in higher blast incidence under such conditions.

Participatory plant breeding of the *in situ* conservation project was employed to blend farmers' knowledge on the genetic pool at community level and farmers' capacity to search for diversity; their skills in seed selection for scientific methods of crop improvement are enhanced. We realized that farmers' selection was based on and effective for visible traits such as plant height, grain colour and days to maturity in segregating bulks. Also the bulk breeding method was easy to adapt to and effective in local conditions. Therefore, selection for yield components was delayed until  $F_{5-6}$  but focused on farmers' known preferred and visible traits. The modified bulk breeding and pure lines from bulk breeding methods (Figure 1) were used as explained by Gyawali et al. 2002. The bulks whose population sizes were large enough to advance the generations were modified based on farmers' preferred traits in the crosses. For example, *Mansara/Khumal 4* was modified on the basis of grain colour in  $F_3$  generation because the farmers preferred *Mansara* grain colour over straw colour. Furthermore, the grain colour was governed by major genes and farmers' selections on the basis of these traits were effective in early generations.

The post-harvest evaluation of selected materials from the remnant seed was extremely important to the PPB programme (Gyawali et al. 2002, 2004c). This was especially true in *Mansara/Khumal 4* cross where collaborating farmers evaluated the remnant seed after harvest for cooking and eating qualities such as taste, softness and flakiness. It was found that the complementary parent *Khumal 4* has contributed to improving the post-harvest quality traits in progenies of *Mansara/Khumal 4* crosses. Farmers' perception on cooking and eating qualities for selected bulks in the present programme was recorded and selected materials were further screened for stress tolerance and yield components. These screenings for agronomic, milling and organoleptic traits have enhanced the populations to meet the breeding goals in PPB programmes.

Sthapit et al. (1996) and Gyawali et al. (2002) demonstrated that PPB could be an option to manage stress in high-altitude rice and drought tolerance in low-altitude rice in Nepal, respectively. Genetic studies of abiotic and associated biotic stresses provide valuable information to PPB. This will enhance the understanding of the stress-tolerance mechanism on one hand and the selection efficiencies of breeders on the other hand. For example, Sthapit et al. (1995) reported that taller plants and better panicle exertion of rices resulted in higher genetic gain to chilling tolerance and resistance to ShBR in high-altitude rices in Nepal. In 2005, in the middle hills of Nepal, more than 100 farmers are involved in evaluating PPB products of  $F_{6-7}$  generations in the *in situ* conservation project in Kaski. Therefore farmers' interest and involvement in PPB has greatly enhanced the deployment of new PPB products on-farm. However the PPB programme of the *in situ* conservation project is not mature enough to assess the impact of PPB on varietal diversity on-farm until 2004 as reported by Joshi et al. 2002 in M 3 and M 9. But the spread of PPB product M 3 and M 9 in high-altitude rice in Kaski district reveals that PPB enhances the varietal diversity on farm. Initially it was argued that PPB would reduce the genetic diversity by replacing local landraces on-farm. However monitoring of M 3 and M 9 for the last 10 years has revealed that more than 60% of rice area is still covered by local landraces. Recently *Lumle-2*, which is one of the breeding materials from earlier PPB work in LARC, has been spreading in PPB villages in high altitudes of Kaski. Farmers adopted *Lumle-2* because this variety has similar cold tolerance, matures together with M 3, is resistant to bacterial sheath brown rot but has superior grain quality. Furthermore, the grain yield of *Lumle-2* is comparable to M3 and M 9.

### **Research gaps**

- Farmers maintain genetic diversity on-farm to meet their niche-specific needs and stress management through diversity deployment is one of the cheapest options for them. However, there is less understanding of how farmers at household and community level deploy genetic diversity to management stresses.
- Stress tolerances are inherent superiority in local landraces over improved varieties which make them competitive in unfavourable as well as favourable production environments but their use in breeding programme to improve the genetic potential of crops to manage abiotic stress is very limited.
- Local landraces become strong competitors when stress tolerances are combined with yield components and market traits (post-harvest quality traits). The non-breeding approaches of adding value to the stress-tolerant rice landraces are poorly exploited.
- The long-term monitoring of the spread of PPB products is very limited and its effects on genetic diversity are not fully understood.

### **Recommendations**

- Use of SAS Construct Analysis is effective for understanding the farmers' local knowledge of diversity deployment for abiotic stress management.
- Participatory plant breeding (PPB) could be an option for using local genetic resources as well as farmers' knowledge and skill to manage abiotic stress through diversity deployment. When abiotic stress becomes extreme, in such conditions, PPB becomes more important.
- The non-breeding approaches of adding values such as market and use values could enhance the agrobiodiversity under abiotic stress conditions.
- The long-term monitoring of PPB products is required to understand the effect of PPB on genetic diversity.

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### **References**

- Bajracharya, J. 2004. Genetic diversity study in landraces of rice (*Oryza sativa* L.) by agromorphological characters and microsatellite DNA markers. PhD Thesis. School of Agricultural and Forest Sciences, University of Wales, UK.
- Central Bureau of Nepal. 2004. Statistical year book of Nepal. National Planning Commission, Nepal.
- Chevalier, J.M. 2005. The Social Analysis System. Carleton University, Ottawa, Canada. <http://www.sas-pm.com> accessed date 01/05/2005.
- Gaines, B.R. and Mildred L.G. Shaw. 2004. Construct Analysis of Social Analysis System. Rep IV 1.0 Personal Version 2004. Centre for Person-Computer Studies, 3635 Ocean View, Cobble Hill, BC V0R 1L1, Canada.
- Gyawali, S., Joshi, K.D. & Witcombe, J.R. 2002. Participatory plant breeding in low-altitude production systems in Nepal. pp 8-11. *In*: Breeding rainfed rice for drought-prone environments: integrating conventional and participatory plant breeding in South and Southeast Asia. Proceedings of a DFID Plant Sciences Research Programme/IRRI Conference, 12-15 March 2002, IRRI, Los Baños, Laguna, Philippines. Department for International Development (DFID) Plant Sciences Research Programme, Centre for Arid Zone Studies (CAZS) and International Rice Research Institute (IRRI), Bangor and Manila. pp. 8-10.
- Gyawali, S., K.D. Joshi, R.K. Tiwari, P. Shrestha, B.K. Joshi, B. Chaudahry, A. Mudwari, B.K. Baniya, A. Subedi, B. Bhandari, M.P. Upadhyaya, M. Tripathi, N. Adhikari, K. Shrestha and B.R. Sthapit. 2004a.



- Participatory Plant Breeding: A strategy of *in-situ* conservation of rice landraces. Paper presented in 2nd National Workshop on *in-situ* conservation of agrobiodiversity, 25–27 August 2004, Nagarkot, Nepal. NARC, LI-BIRD and IPGRI.
- Gyawali, S., B.R. Sthapit, R. K. Tiwari, B. K. Joshi and B. Bhandari. 2004b. *Jethobudho* landrace enhancement. II. A participatory evaluation for agronomic traits. Proceedings of the 2nd National Workshop on *in-situ* conservation of agrobiodiversity, 25–27 August 2004, Nagarkot, Nepal. NARC, LI-BIRD and IPGRI.
- Gyawali, S., B. Bhandari, R. Bhandari, J. Bajracharya, M. Tripathi and B.R. Sthapit. 2004c. *Jethobudho* Landrace Enhancement III: Post harvest quality traits. Proceedings of the 2nd National Workshop on *in-situ* conservation of agrobiodiversity, 25–27 August 2004, Nagarkot, Nepal. NARC, LI-BIRD and IPGRI.
- Joshi, K.D., B.R. Sthapit, R.B. Gurung, M.B. Gurung and J.R. Witcombe. 1997. Machhapuchhre 3 (MP-3), the first rice variety developed through a participatory plant breeding approach released for mid to high altitudes of Nepal. *IRRN* 22(2):12.
- Joshi, K.D, B.R. Sthapit and J.R. Witcombe. 2001. How narrowly adapted are the products of decentralized breeding?: The spread of rice varieties from a participatory plant breeding programme in Nepal. *Euphytica* 122(3): 589–597.
- Joshi, K.D., B.R. Sthapit, M. Subedi and J.R. Witcombe 2002. Participatory plant breeding in rice in Nepal. Pp. 239–268 *in* Farmers, Scientists and Plant Breeding Integrating Knowledge and Practice (David A. Cleveland and Daniela Soleri, eds.). CABI Publishing, UK.
- Rana, R.B. 2004. Influence of socio-economic and cultural factors on agrobiodiversity conservation on-farm in Nepal. PhD Thesis. University of Reading, UK.
- Sthapit, B.R. and J.R. Witcombe. 1998. Inheritance of tolerance to chilling stress in rice during germination and plumule greening. *Crop Science* 38(3):660–665.
- Sthapit, B.R., P.M. Pradhanang and J.R. Witcombe. 1995. Inheritance of field resistance to sheath brown rot disease in rice. *Plant Disease* 79(11):1140–1144.
- Sthapit, B.R., K.D. Joshi and J.R. Witcombe. 1996. Farmer participatory cultivar improvement. III. Participatory plant breeding, a case of high altitude rice from Nepal. *Experimental Agriculture* 32:479–496.
- Sthapit, Bhuwon, Krishna Joshi, Ram Rana, Madhusudan Upadhaya, Pablo Eyzaguirre and Devra Jarvis. 2001. Enhancing biodiversity and production through participatory plant breeding: setting breeding goals. *In* An exchange of experiences from South and South East Asia: Proceedings of the International Symposium on Participatory Plant Breeding and Participatory Plant Genetic Resource Enhancement, Pokhara, Nepal, 1–5 May 2000. Participatory Research and Gender Analysis Program, Coordination Office, International Center for Tropical Agriculture, Cali, Colombia. 459 p.
- Witcombe, J.R., K.D. Joshi, S. Gyawali, A.M. Musa, C. Johansen, D.S. Virk and B.R. Sthapit. 2005. Participatory plant breeding is better described as highly client oriented plant breeding. I. Four indicators of client orientation in plant breeding. *Experimental Agriculture* 41:1–21.

## **Use of native and introduced maize diversity to improve cold tolerance in Andean maize**

*Ricardo Sevilla*

### **Introduction**

Low temperature is the most important limiting factor for crop production in the Andean highlands. It shortens the growing season, reduces the productivity and frequently causes death of plants because frost can occur at any stage of plant development, from germination to harvest. The damage is economically very important because for many people—about 10 million small farmers of the Andean countries—maize and a few other crops are the only sources of income and in many areas the products of those crops are all they have to eat.

As the Peruvian people have migrated from the rural farms to the cities at a very high rate, demand for food in the cities has grown steadily, resulting in unsustainable changes in the systems of production because the farmers are shifting from self-consumption to market production. One such change is the tendency to plant earlier. However, the Peruvian maize varieties, though well adapted to the cold highlands, do not have enough cold tolerance at the first stage of development, and consequently are vulnerable in early plantings.

A totally different genetic system governs cold tolerance in the early and late stages of development. Posch (1994) reviewed the literature about cold tolerance heredity in maize. Some researchers have found evidence for additive gene effects (Grogan 1970; Mock and Eberhart 1972). Others have found non-additive effects to be equally important. Eagles and Hardacre (1979) in New Zealand analyzed the genetic variance of the germination ability and emergency at 10°C. They concluded that percentage germination and seed weight were predominantly maternal and that there exists a considerable amount of dominance variance for rapid emergence at 10°C.

The objectives of the research are (1) to build the cold-tolerant base population of maize with Peruvian and foreign germplasm, (2) to improve cold tolerance in the early stages of development, and (3) to improve productivity and other important agronomic characteristics of the new varieties without losing the Andean maize ear avoidance mechanisms.

### **Materials and methods**

This research has been done in three stages. In the first stage all the known cold-tolerant maize germplasm was brought to Peru. In the second stage that foreign germplasm was evaluated, *per se* and in crosses with Peruvian highland varieties. In the third stage, intrapopulation selection was carried out, maximizing recombination. In addition, the best Mexican, Bolivian and Peruvian germplasm was tested for cold tolerance at the late stage of development.

The main activities were done at the Sierra Regional Development Institute of UNALM (Universidad Nacional Agraria La Molina, IRD-Sierra). The experimental station is located in Jauja (Mantaro Valley) at 3340 meters above sea level (masl). The research started in 1977.

### **Cold-tolerant germplasm**

In the 1980s researchers recognized that cold tolerance was a very complex characteristic and the genetic basis of the components was poorly understood, so it was necessary to get every source of cold-tolerant germplasm. Six populations were formed by integrating the foreign with the Peruvian germplasm. Details of the germplasm integrated in the six populations were given by Sevilla (1988, 1995).

1. Native germplasm from Peru, adapted to the highlands, earlier, with large floury kernels. Also included in this group were one Mexican variety (Cacahuacintle) and one from

Guatemala (Huatayán Xela). They were planted in 1977 in the IRD-Sierra. A very intense selection was done pollinating only the plants that were early, healthy and free of cold damage. They were selfed and with the same pollen crossed to about 5 plants of the same population. In 1978 two experiments were conducted to test half-sib families. Two replications were planted in IRD-Sierra and two in Huancayo (3200 masl).

2. Another good source of cold-tolerant germplasm is the material that CIMMYT used for the Cooperative Andean Maize Project. This material was tested in the highlands of Peru and 19 early populations were selected. Besides the CIMMYT highlands populations: Pool 1, Pool 2, Pool 4 and Pool 5 were tested in the IRD-Sierra in 1978 together with local varieties as checks. The CIMMYT's Pool 2 and Pool 4 yielded 12% more than the best local test. However the texture, shape and size of the grain were different from the local varieties and they have some bad characteristics such as lodging susceptibility. Considering the reports of Miedema (1979), Hardacre and Eagles (1979) and Eagles and Brooking (1981), Pools 4 and 5 were selected because of their proven cold tolerance, but Pool 5 was discarded because of its yellow colour and dent texture of the grain. The population formed by crossing 11 Peruvian varieties and the Compuesto Cacahuacintle to 19 populations of the CIMMYT Cooperative Andean Maize Program is Population 2 (TF). The F1 was selfed in 1978 and 752 S1 inbred lines were planted in 1979 to evaluate the germination ability under field stress conditions.

3. In 1967 a project to select cold-tolerant germplasm was started in Cornell University. The methodology was set to detect genetic material with the ability to germinate at low temperature (7–13°C), ability to grow and develop at low temperature and the ability to withstand –2°C for 15 hours at the seedling stage. In the following years, experiments were sent to several countries to test the selected material in field conditions. The Peruvian experiment was planted in the IRD-Sierra. All the 16 Mexican populations were selected in the adaptation test, but only one entry from Pakistan, one from Russia, one from Canada and one from the United States were used. This germplasm is called in this paper 'Grogan material' in reference to the scientist who coordinated that project. The Grogan material was crossed to the Peruvian highly adapted variety San Gerónimo and the hybrids were backcrossed once to San Gerónimo. In the field where the backcrosses with San Gerónimo were produced, 158 full sib families were evaluated and compared with the Peruvian variety.

4. To reduce the lateness of the Blanco del Cuzco variety (BU), it was crossed to San Gerónimo (SG) and recombined for several generations. Selfed S1 progenies were tested per se during two cycles of recurrent selection. The synthetic BU x SG was formed by recombining the best lines. This fourth population was named PMS-636.

5. The fifth source of cold-tolerant germplasm was the Compuesto Choclero Precoz. The composite was formed in 1978 with almost all large white floury kernel varieties from Latin America. The composite was recombined and selected by mass selection in 1980. Ear to row selection started in 1981.

6. The Canadian material came from the Plant Research Centre in Ottawa, Ontario. Sixteen different populations were tested and crossed in the IRD-Sierra to PMS-636, a Peruvian improved variety. PMS-636 was formed by recombining in a synthetic the S2 lines from Blanco del Cuzco x San Gerónimo that survived a very severe frost. The best plants of each F1 progeny were pollinated. In 1991, 200 S1 line progenies were planted and the best plants were self-pollinated after a very heavy frost. In 1992, 336 S2 lines were planted and again a very heavy frost destroyed the crop completely. The crop was open pollinated. At harvest only ears from the survivor plants were collected.

The Canadian  $\times$  PMS-636 hybrids were selfed until S2. The S2 lines were being evaluated per se in an experiment when a frost destroyed the crop. A total of 177 ears that came from seven Canadian populations crossed to PMS-636 were saved. An ear to row selection project was started with those ears.

### **Selected LAMP accessions**

The Latin American Maize Project (LAMP) evaluated maize accessions (LAMP 1997) from 12 countries' germplasm banks in five different homologous areas (HA). The HA 4 corresponds to the accessions adapted to higher altitude than 2600 masl. Fourteen accessions from Mexico, eight from Peru, six improved Peruvian varieties, five crosses between Peruvian accessions by the best Bolivian highlands tester and four Peruvian populations from the Cold Tolerance Project were evaluated in two planting dates: early November (normal) and late December (stress cold environment). Both planting dates suffered from cold damage in the late stage, affecting the ear already formed. Seventeen ear morphological characteristics were evaluated besides the score of ear cold damage. A correlation coefficient was calculated between those characteristics and the score of ear cold damage (Evaristo 1995).

### **Results**

In the first population 19 families were selected for yield, earliness and ear quality. The inbred S1 lines of each selected family were intercrossed in 1980, and recombined in 1981 and 1982. The synthetic was named Population A (TF)- Sint 1.

In the second population results showed big differences between lines. The best one was derived from a cross where (Morado  $\times$  UNCEF 242  $\times$  Pool 4) was the female. None of the 18 lines obtained with Chihuahua  $\times$  Titicaca as the male parent germinated. Lines from Puebla opaco  $\times$  Barraza germinated fairly well, but there were large differences between lines from the same cross. Very few populations had the capacity to transmit to their offspring the ability to germinate in that environment. The only population that apparently has that capacity is (Morado  $\times$  UNCEF 242  $\times$  Pool 4). When it was used as female, the average germination percentage of their lines from 5 crosses was 53.9%; and when it was used as male, germination was 54.3% (data not shown). The larger values correspond to two crosses using (Morado  $\times$  UNCEF 242  $\times$  Pool 41) as the female parent (Table 1).

Table 1 shows large differences between reciprocal crosses. As the evaluation was about progenies of the inbred lines, where the female and male parents are the same, the differences must be due to cytoplasmic effects rather than single maternal effects. In 1982, 103 lines selected for the ability to germinate in cold environments were planted. Twenty-two lines were selected and intercrossed. The generated synthetic was named Population B (TF) Sint 1.

In 1984, 287 S2 lines were obtained by selfing the best plants of the 22 S1 lines. In 1985, the Syn 1, 22 S1 lines, 287 S2 lines and 24 Peruvian landraces were tested under early planting conditions in the IRD-Sierra. The freezing weather destroyed the experimental material, but the ability to germinate could be evaluated before the frost. The germination percentage and seedling vigour of that material is shown in Table 2. The average of the S1 lines was 87%, the synthetic value was 77% and the S2 average was 79%. Within the lines that formed the Sint 1, the lines with (Cacahuacinte  $\times$  San Gerónimo) as one parent were the best: 88.5% average. The Peruvian landraces showed the lowest average values: germination 64% and seedling vigour 1.52. Selected lines were intercrossed to form the Population B (TF) Sint 2.

**Table 1. Field germination percentage of S1 lines from Peruvian x foreign crosses.**

| Female                                | Male                             | No. tested lines | % germination |        |
|---------------------------------------|----------------------------------|------------------|---------------|--------|
|                                       |                                  |                  | Mean          | +/-1SD |
| C. Cacahuacintle (M)                  | × C. Cacahuacintle (M)           | 7                | 41.70         | 17.50  |
| C. Cacahuacintle (M)                  | × (Chihuahua × Titicaca) (M × P) | 6                | 0             | 0      |
| (Chihuahua × Titicaca) (M × P)        | × C. Cacahuacintle (M)           | 5                | 26.80         | 10.50  |
| C.R. Piscorunto (P)                   | × (Chihuahua × Titicaca) (M × P) | 12               | 0             | 0      |
| C.R. Piscorunto (P)                   | × (Puebla Opaco × Barraza) (M)   | 20               | 46.40         | 14.1   |
| C.R. SGI (P)                          | × (Puebla Opaco × Barraza) (M)   | 3                | 44.0          | 17.40  |
| (Puebla Opaco × Barraza) (M)          | × C. Cacahuacintle (M)           | 26               | 54.40         | 32.50  |
| (Sintético BU × SG) (P)               | × C. Cacahuacintle (M)           | 8                | 1.00          | 1.85   |
| (Sintético BU × SG) (P)               | × (Puebla Opaco × Barraza) (M)   | 8                | 10.50         | 21.20  |
| (Puebla Opaco × Barraza) (M)          | × (Sintético BU × SG) (P)        | 25               | 53.00         | 29.10  |
| (Morado × UNCEF 242 × Pool 4) (F × C) | × (C.R. SGI) (P)                 | 4                | 70.00         | 2.80   |
| (Morado × UNCEF 242 × Pool 4) (F × C) | × Bco. Am. Precoz (P)            | 8                | 65.80         | 18.20  |

M = Mexican; P = Peruvian; F = French; C = CIMMYT.

**Table 2. Germination percentage and seedling vigour of Population B (TF) Synt-1, their S1 and S2 lines, S1 from (Grogan × SG) and Peruvian landraces.**

| Population         | No. of entries | % germination | Seedling vigour <sup>†</sup> |
|--------------------|----------------|---------------|------------------------------|
| B (TF) Synt – 1    | 1              | 77            | 1.75                         |
| B – S1             | 22             | 87            | 2.08                         |
| B – S2             | 287            | 79            | 1.73                         |
| (Grogan × SG) – S1 | 20             | 84            | 2.06                         |
| Peruvian races     | 24             | 64            | 1.52                         |

<sup>†</sup> 1 = very bad; 5 = very good.

In testing the Population 3 materials it was shown that in general the foreign germplasm tends to produce taller plants in crosses with San Gerónimo, which are later and very much susceptible to rust. The Mexican material is later than San Gerónimo, it has many tillers and lodging susceptibility; but it is the best germplasm to induce rust resistance, yield potential and cold tolerance. In the trial that tested 62 backcrosses × San Gerónimo, the check variety (San Gerónimo) ranked 63rd; however it was the earliest entry. Only PI-267169 from Russia and the North American single hybrid W59E × W10 were similar in earliness to San Gerónimo.

In 1980 the 62 backcross progenies were planted and 656 S1 lines were obtained. The following years they were evaluated per se and the five best looking ears from 104 lines were selected. The 520 ear progenies were planted in 1982 to initiate the half sib family selection. In the second half sib family selection cycle the selected material was backcrossed again to San Gerónimo. The resulting population was named Population C. In the third cycle the female population was formed with 535 ear progenies from population C, 31 full sib (FS) families from Population A (TF) Sint 1; F1 FS families from Mexican × San Gerónimo; 16 FS families from Peruvian racial composites and 10 FS sib families of PI-267169 (Russia) × San Gerónimo.

In 1985 selected 234 HS families of Population C were planted together with 50 FS families of Cacahuacintle × San Gerónimo. The system permits us to incorporate as female plots all the materials that are evaluated phenotypically in the nursery fields. The male for every cycle is formed with seed of the selected ears of the preceding cycle. In each cycle, two out of four replications were planted in October and two in December (stress season).

In 1986 Population C was crossed to PMS-636 because it still did not have a good level of adaptation and ear quality. The resulting population was named Population D.

PMS-636 was improved and recombined in four ear to row selections. In each cycle, two out of four replications were planted in October and two in December. As December is the stress season because maize is exposed to the cold weather in June and July, the resulting selected variety is adapted to a late planting season.

The Compuesto Choclero Precoz was selected by ear to row selection during eight cycles. In 1988 it was crossed to Population D to produce Population E. After one cycle of mass selection it was crossed to PMC-584, a late Cuzco-type variety, to form Population Ch (Ch=choclo or green corn).

The ear to row selection of the population formed by crossing Canadian material with PMS-636 passed through two cycles of selection. The resulting improved population (PMG-639) is by now the earliest variety; it is earlier than San Gerónimo and it has maintained the Andean type of grain. A summary of the selection and recombination work to form and select the populations to obtain the improved varieties is shown in Table 3.

**Table 3. Building the base populations and selection of the cold-tolerant maize varieties.**

| Year | Population        |                     |                     |                   |                      |                     |
|------|-------------------|---------------------|---------------------|-------------------|----------------------|---------------------|
|      | A <sup>†</sup>    | B                   | C                   | BU × SG           | C. CH. P.            | G                   |
| 1978 | Peruvian HS       | Perú × CIMMYT       | Grogan × SG         | ⊗                 | Crossing white flour |                     |
| 1979 | ⊗                 | ⊗                   | FI × SG             | Line per se       | Recombination        |                     |
| 1980 | Pob A (TF) Sint I | Seedling evaluation | ⊗                   | (BU × SG) Sint II | MS                   |                     |
| 1981 |                   | Seedling selection  | Line per se         | Recombination     | SMH (C.1)            |                     |
| 1983 |                   | Pob B (TF) Sint 1   | HS × SG / SMH (C.1) | SMH (C.1)         | SMH (C.3)            |                     |
| 1984 |                   | Recombination       | SMH (C.2)           | SMH (C.2)         | SMH (C.4)            |                     |
| 1985 |                   |                     | SMH (C.3)           | SMH (C.3)         | SMH (C.5)            |                     |
| 1986 |                   |                     | SMH (C.4)           | SMH (C.4) PMS-636 | SMH (C.6)            |                     |
| 1987 |                   |                     | Pob D               |                   | SMH (C.7)            |                     |
| 1988 |                   |                     | SMH (C.1)           |                   | SMH (C.8)            |                     |
| 1989 |                   |                     | SMH (C.2)           |                   | Pob E (MS)           | Canadian × PMS-636  |
| 1991 |                   |                     | SMH (C.4)           |                   | Recombination        | ⊗                   |
| 1992 |                   |                     | SMH (C.5)           |                   | Pob E SMH (C.1)      | Line per se         |
| 1993 |                   |                     | SMH (C.6)           |                   | Pob E × PMC-584      | ⊗                   |
| 1994 |                   |                     | SMH (C.7)           |                   | Pob CH (SMH) (C.1)   | SMH – (C.1)         |
| 1996 |                   |                     | SMH (C.9) PMD-638   |                   | SMH C.3              | SMH – (C.2) PMB-639 |

<sup>†</sup> C=Cycle; Pob = population; TF=cold tolerant; SMH=ear to row selection; HS=half sib progeny; MS=mass selection; ⊗=selfing; SG=San Gerónimo; BU=Bianco Cuzco.

Evaluation of the cold tolerance ear avoidance mechanism was made by correlating 17 different ear morphological characteristics with the ear cold damage value. Five ear characteristics correlated with cold damage. Results are shown in Table 4. Only three ear characteristics—external husk leaf width, ear diameter and grain length—are negatively

correlated with cold damage, i.e. the larger the characteristic value, the less damage. Figures 1 and 2 show results and interpretation of this specific research.

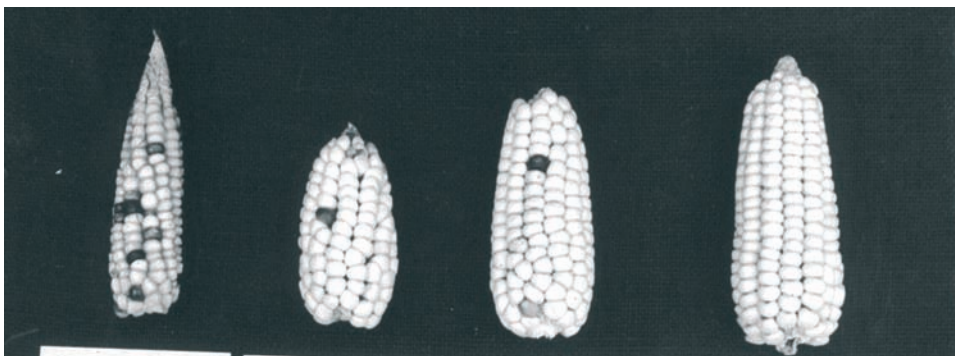
**Table 4. Correlation coefficients of five ear characteristics with frost damage values that showed significance, Jauja 1992.**

| Ear morphological characteristics | Bolivian       |                | Peruvian |        | Mexican |       | Peruvian × Bolivian |        | Cold-tolerant vars. |    |
|-----------------------------------|----------------|----------------|----------|--------|---------|-------|---------------------|--------|---------------------|----|
|                                   | N <sup>†</sup> | S <sup>†</sup> | N        | S      | N       | S     | N                   | S      | N                   | S  |
| External husk leaf width (cm)     | 0.66**         | 0.88*          | 0.41**   | 0.40** | 0.32**  | 0.29* | ns                  | 0.51** | —                   | ns |
| No. husk leaves                   | 0.69**         | ns             | 0.21**   | ns     | ns      | ns    | 0.28*               | ns     | 0.56*               | ns |
| Total husk leaf width             | ns             | ns             | 0.15*    | -0.33  | —       | —     | ns                  | ns     | ns                  | ns |
| Ear diameter                      | ns             | —              | —        | —      | —       | —     | —                   | —      | -0.4**              | —  |
| Grain length                      | —              | —              | 0.41**   | —      | 0.39**  | —     | 0.30**              | —      | —                   | —  |
|                                   | 0.57*          | —              | 0.23*    | —      | 0.37**  | —     | —                   | —      | —                   | —  |

<sup>†</sup> N=Normal planting data; S= Stress planting date; *P*=0.01.



**Figure 1. Cold-tolerance avoidance characteristics in maize ear husks: number of husk leaves (left four husks), compactness (centre four husks), husk colour (right four husks).**



**Figure 2. Cold tolerance avoidance characteristics in maize ear shapes: (from left) Guanajuato 140, San Gerónimo, Population D, Population E (cold tolerant).**

### **Discussion**

Assembling all the cold-tolerant germplasm in a few populations does not ensure that all the genes have been maintained in the improved populations. Also the small cold-tolerance values in Peruvian germplasm found in some research does not mean that there are no genes for cold tolerance in the Peruvian germplasm. Often the genes for stress tolerance are in low frequency in genetic populations evolved in stress environments (Sevilla and Holle 2004). The main reason for this situation is the nature of the tolerance characteristics. They are very complex features, combining avoidance morphological mechanism with real tolerance as defined by Levitt (1980). As stress occurs at different times and in different ways and environments, the natural selection or artificial selection is not isodirectional. Genes are maintained across populations but at a low frequency. Keim and Gardner (1984) estimated the genetic variation for cold tolerance in selected and unselected maize populations. The additive genetic variance increases from the 1<sup>st</sup> to 4<sup>th</sup> cycle of selection. They explained that by assuming that the alleles for cold tolerance may have been at low frequency in the parent population. With selection, the frequency of alleles for cold tolerance would increase toward the value of 0.5 when additive genetic variance would be at a maximum.

As the tolerant characteristics in the first stage of development are mainly additive, Mock (pers. comm.) suggested a programme of recurrent selection for the Peruvian maize in the highlands. Evaluation of the selection response made in three populations—Population C, PMS-636 and Compuesto Choclero Precoz—shows that the genotype  $\times$  environment interaction was very high. The only case where yield improvement was positive was in PMS-636 in the stress season (Gamarra 2004). PMS-636 was selected in the average of the normal and stress seasons. However the three resulting populations were earlier and smaller as was expected, taking into consideration the tendency of adaptation for non-adapted germplasm to the highlands (Sevilla and Salhuana 1970).

The real value of the Peruvian highland germplasm is the ear avoidance mechanisms. They have been created in the evolution process to adapt maize (Greenblatt 1985). They have to be maintained because they are real defenses against the cold weather. However the maize in the highlands has to have a broader base of cold tolerance. Still there is no good methodology for field evaluation of cold tolerance at the first stage of development. Percent of germination, rapid emergency and seedling vigour have low heritability when they are evaluated in the field and there are many opportunities for escape. This is a typical case where marker-assisted selection is recommended. Only the plants or families with the specific markers must be selected from plants with Peruvian ear and grain type.

The Peruvian germplasm complements the temperate germplasm. Data from Hardacre and Eagles in New Zealand (1979) show that the larger the seed the greater the ability to grow autotrophically at 13°C. Three Peruvian races (San Gerónimo, Huancavelicano and Confite Puneño) had a greater autotrophic growth capacity than other non-Andean races. However Eagles and Brooking (1981) showed that San Gerónimo had the most cold damage, slower germination and the lowest emergence percentage. When the seedlings begin to depend on energy from photosynthesis they become more sensitive because photosynthesis is affected by temperature (Eichelberger 1990).

The question about the persistence of the cold tolerance genes in the recombined and improved populations has been answered out of Peru. Brandolini et al. (2000) presented data from the evaluation of some Peruvian improved cold-tolerant populations compared with Bolivian and Peruvian accessions and several check Italian and North American hybrids. PMS-636 showed, in the heterotrophic growth trial, the lowest germination index value S/C (stress/normal) expressed in number of days, compared with 19 Peruvian and Bolivian accessions and six checks. A lower value means better cold adaptation and rapid germination. In the heterotrophic and autotrophic trial in the field, the emergence percentage of Compuesto Amarillo de Ancash had the largest treatment in the stress evaluation and in the S/C value. Population D and Population E were statistically superior in plant growth



rate in stress environment and in S/C value than all accessions and three checks, except for PMS-636, Confite Puneño and Apuc 269, an elite accession of the Piscorunto race.

The CIMMYT Population 5 has been used to generate temperate hybrids with more tolerance to the cold weather in the first stage of development (Hardacre and Greer 1989; Hardacre et al. 1990). However, Population 5 was not used in Peru because of its sharp difference in type of ear and grain. If marker-assisted selection is used it is possible to use that source as well as other sources. In northern Europe there must be good sources of cold tolerance in the first development stage. Many countries have been planting maize for many years in the early spring; it is surprising not to find good sources of this characteristic. It is necessary to build a stronger relationship between temperate and Andean countries to deepen the knowledge of this complex characteristic.

## References

- Brandolini, A., P. Landi, G. Monfredini and F. Tano. 2000. Variation among Andean races of maize for cold tolerance during heterotrophic and early autotrophic growth. *Euphytica* 111:33–41.
- Eagles, H. and A. Hardacre. 1979. Genetic variation in maize (*Zea mays* L.) for germination and emergence at 10°C. *Euphytica* 28:287–295.
- Eagles, H. and R. Brooking. 1981. Populations of maize with more rapid and reliable seedling emergence than cornbelt dents at low temperatures. *Euphytica* 30:755–763.
- Eichelberger, K.D. 1990. Evaluation of progress from selection for cold and freeze tolerance in maize. PhD thesis, University of Nebraska, USA.
- Evaristo, J. 1995. Características morfológicas de la mazorca de maíz (*Zea mays* L.) asociadas a la tolerancia al frío en germoplasma peruana y foreneo. Tesis Mg. Sc. UNA La Molina, Lima, Perú.
- Gamarra, G. 2004. Gamamcoa genética por selección mazorca-hilera modificada en tres poblaciones de maíz amiláceo. Tesis Mag Sc, UNA La Molina, Lima, Perú.
- Greenblatt, I. 1985. The ear of maize as a heat conserving device. *In: Northeastern Corn Improvement Conference*. University of Massachusetts, USA.
- Grogan, C. O. 1970. Genetic variability in maize for germination and seedling growth at low temperatures. *Proceedings of the Annual Corn Sorghum Research Conference* 25:90–98.
- Hardacre, A. and H. Eagles. 1979. Comparisons among populations of maize growth at 13°C. *Crop Science* 20:780–784.
- Hardacre, A. and D. H. Greer. 1989. Differences in growth in response to temperature of maize hybrids varying in low temperature tolerance. *Australian Journal of Plant Physiology* 16:181–187.
- Hardacre, A., H. Eagles and C. Gardner. 1990. Genetic variation for frost tolerance of maize (*Zea mays* L.) seedlings. *Maydica* 35:215–219.
- Keim, K. and C. Gardner. 1984. Genetic variation for cold tolerance in selected and unselected maize populations. *Field Crop Research* 8:143–151.
- LAMP. 1997. Latin American Maize Project. Final Report. W. Salhuana, R. Sevilla and S. Eberhart, editors.
- Levitt, J. 1980. Responses of plants to environmental stresses. Vol. I. Chilling, Freezing and High temperature stresses. Academic Press.
- Miedema, P. 1979. Potential use of CIMMYT gene pools material for improving low-temperature adaptation in *Zea mays*. *Euphytica* 28:661–664.
- Mock, J. and S. Eberhart. 1972. Cold tolerance in adapted maize populations. *Crop Science* 16:466–469.
- Posch, J. S. 1994. Evaluation of maize inbreeds for cold tolerance when grown in controlled field environments. PhD thesis dissertation. University of Nebraska, USA.
- Sevilla, R. 1988. Selección para tolerancia al frío en maíz. En: III Seminario: Mejoramiento para tolerancia a factores ambientales adversos en el cultivo de maíz. Ed: B. Ramakrishna. PROCIANDINO. Setiembre 1987. Quito, Ecuador.
- Sevilla, R. 1995. Germoplasma foraneo de maíz tolerante al frío en los primeros estados de desarrollo para daptar las variedades de la sierra del Perú a siembras tempranas. III Reunión Latinoamericana de Maíz. Pp. 149–166. Cochabamba-Santa Cruz, Bolivia.
- Sevilla, R. and M. Holle. 2004. Recursos Genéticos Vegetales. Ed. Luis León As. Lima, Peru.
- Sevilla, R. y W. Salhuana. 1970. Comportamiento de semilla de la variedad Blanco Urubamba producida en cuatro ambientes distintos de la sierra del Perú. *Fit Lat Vol 8, No. 1.*

## **Synthesis – Cold stress**

The reports from Hungary, Bolivia and Nepal have common themes that parallel those for drought stress. These themes include the definition of cold stress, analysis of the plant's response, and of farmers' management practices. It is important to characterize the kind and degree of cold stress (e.g. as chilling, frost, freezing and hail) in order to analyze plant responses. For example the primary lesions on quinoa in Bolivia (i.e. broken leaves from hail) have different effects on the plant than secondary lesions (i.e. infection or stunted growth after hail damage). It is also important to define the difference between cold tolerance (biochemical mechanisms that allow a plant to respond to a stress), and cold avoidance (morphological and phenological characteristics, such as early maturity that allow the plant to escape from a stress).

As with drought stress, the role of farmers' practices to manage cold stress requires attention. Specifically, it is important to understand practices based in traditional knowledge, and the impacts of changing market and cultural forces on such knowledge systems. Examples from Bolivia demonstrate a close link between preventing frost damage and community practices. In this study, an elected community member is charged with the responsibility of protecting crops from frost and hail by shooting fireworks and making smoke near the crops. Observations of similar practices in Hungary raised the question of the potential scientific bases of ritual practices. Nonetheless, in contrast to these examples of traditional practices with deep historical roots, commentaries from Peru and Hungary suggest that changing market demands are also changing farmers' choices of planting material. Farmers in these places seek more cold-tolerant varieties owing to the push to enter into the market earlier in the season and thereby obtain higher prices.

## Group Discussion Summary: Issues and Future Directions

*A. Keleman, T. Brown and D.I. Jarvis*

Group discussion addressed the adaptation of local crop varieties to the climatic and edaphic stresses in particular systems, the genetic variation for stress responses, and the role of farmer management practices at various scales. This section summarizes salient points emerging from these discussions.

An understanding of strategies of tolerance or avoidance of stress that may be present in traditional varieties and diversity management will require attention to changes in stress and plant conditions over space and time. More than one variety can manage a single stress, different stresses can affect the same plant at different times, and a plant's life cycle and maturity stage affect its response to stress. Potential research questions for analyzing these relationships included:

- How should one define a stressed environment across different geographical locations and environmental contexts?
- How does the damage from abiotic stress make a crop more at risk from other stresses, such as pests and disease?
- How are strategies affected by different units of analysis (e.g. a single crop vs. an agroecosystem), or in different kinds of cropping systems (e.g. vegetables vs. grains; annuals vs. perennials)?
- At what scale must diversity be present in order to be used optimally and maintained? At a regional scale? Or within-field? How do community seed networks affect the way in which diversity is available to farmers to cope with spread?

Large-scale abiotic changes—especially in global climate patterns—present a threat to crop genetic diversity. The pace of change could be such that genes for stress tolerance were disappearing faster than they can evolve into multigenic tolerant genotypes. Questions posed in this vein include:

- How should one determine a hierarchy of abiotic stress? How does the impact of stress vary among farmers and regions? How should acuteness, breadth of impact, forms of available mediation, and other criteria be used to prioritize abiotic stress research and extension?
- Which varieties are disappearing in a given context? Why are some varieties continuing to survive under changing conditions while others are not?
- Is there sufficient genetic variability in a given crop for PPB to be effective?

The problems caused by abiotic stress in a given farming system occur along with other economic, political and environmental pressures. Subsistence agriculture and large-scale commercial production represent only the extreme ends of the spectrum, and most small-scale farmers manage their crops for a wide variety of uses and market values and in the face of many challenges. Research questions included:

- To what extent are the various kinds of stress a problem on-farm? In the face of these problems, is crop diversity the leading option for stress management, or is it simply another element of the farming system?
- To what extent is it possible to separate genetic changes induced by farmer management from those imposed by natural pressures?
- How do other farming practices—such as intercropping—play a role in mediating abiotic stress? How do such practices influence how farmers maintain or select genetic diversity?

Finally, questions that address farmer management in the context of broader patterns of environmental change include:

- How are changing climatic and economic factors affecting traditional knowledge for managing stress?
- In what systems is there a potential for ameliorating the rapid processes that cause loss of diversity such as increased used by farmers of the diversity in genebanks?

Participants' presentations were followed by three working-group sessions on abiotic stress issues. The first session sought to define key questions on the link between abiotic stress and genetic diversity. The second discussion examined questions about farmers' management of genetic diversity under stress conditions. Finally, the third session explored the possible intensification of abiotic stresses due to land degradation and climate change.

The final working-group period addressed the range of abiotic stresses as they afflict crop production, and criteria for ranking them. Major abiotic factors on-farm included not only drought, cold and salinity, but also low soil fertility (characterized as nitrogen and phosphorus deficiency), boron and iron deficiency, boron toxicity, soil acidity, erosion, waterlogging, and climatic disasters such as flooding and hurricanes. Drought was ranked as the highest priority, followed by low soil fertility, soil acidity and cold stress. Suggested criteria for ranking stresses included the risk to poor farmers, the extent of potential damage to agricultural production, the area of the land affected, and the likelihood that one stress might worsen other biotic and abiotic stresses.

Based on the working group output and the discussions surrounding it, participants drafted a minimum set of questions. These questions are listed in Table 1.

**Table 1. Draft minimum set of questions on abiotic stress.**

| <b>Characterizing stress and plant response</b>   |   | <b>Genetic variation for tolerance</b>   |  |
|---|---|--|--|
| <b>Researcher</b>   | <b>farmer Knowledge</b>   | <b>Researcher</b>  | <b>Farmer knowledge</b>  |
| How should stress be characterized and measured?  | What are farmers' perceptions of stress? What markers do farmers traditionally perceive as signs of stress? | Within a given crop's population structure, what is the genetic diversity for tolerance?   | How do farmers assess stress tolerance? How do they assess variation in stress tolerance?  |
| What are the effects on diversity management of multiple stresses and their interaction with each other?<br>To what extent is stress predictable and variable? What is the nature of its predictability or its variability?<br>What are the changes in the intensity of stress over time? | What limits access to associated knowledge to cope with stress?   | Is there a genetic relationship between stress-response components and other traits? If so, what is the nature of this relationship?<br>What are the genetic consequences of adapting to extreme stress? | How do farmers make trade-offs between stress tolerant characteristics and other traits?<br>What limits the access to genetic diversity to cope with stress? |
| How are the above questions affected by temporal and spatial scales?  |   | How does microenvironment diversity relate to genetic variation?   |  |
|   |   | What are the roles for genetic manipulation (participatory plant breeding, control of gene flow, etc.) to improve adaptation?  |  |

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