Prospects of success of biotechnological approaches for improving tolerance to drought stress in crop plants

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Abiotic stresses such as drought, temperature, salinity and others generally reduce crop productivity. The abiotic stresses are location-specific, exhibiting internal variation in occurrence, intensity and duration. These could occur at any stage of plant growth and development which represent the dynamic nature of crop plants and their yield. A few transgenics in tobacco and rice overproducing osmolytes and stress proteins have been produced and tested for tolerance to salt and drought stress in the vegetative stage. The genes being manipulated are not mechanistically linked with the process of yield formation. Using biotechnological approach it is difficult to foresee abiotic stress-resistant transgenics growing in the farmer's field in the near future. It is suggested that breeding for drought tolerance requires the identification of traits for phenology and those contributing to yield so that these can be pyramided using conventional means.

THERE is a serious concern for food security of developing countries including that of India for the following reasons: (i) Increasing food demand for the rapidly bourgeoning population, which will be further enhanced due to improved economic growth, (ii) Stagnating or declining productivity in high productivity regions, often described as 'Green Revolution' fatigue, and (iii) Increasing vulnerability to agriculture as a result of potential climate change.

Therefore, there is a conscious effort to improve production from areas which are commonly exposed to abiotic stresses, such as drought, temperature, salinity, alkalinity, waterlogging and nutrition. We are confronted with the following major problems: (i) Almost all abiotic stresses adversely influence growth, induce senescence leading to death or reduced crop yield. Is there a common mechanism through which abiotic stresses cause these effects? (ii) What are the target traits which can be amenable to genetic engineering (biotechnology) for improving tolerance or resistance to abiotic stresses? (iii) Are expression and selection of tolerance for abiotic stresses in tissue culture or transformed plantlets maintained at all stages of development? (iv) How can plant processes leading to grain yield be manipulated by a few genes? Since it is not possible to address the problems of many abiotic stresses in a relatively short article, we largely confine here to the problem of drought.

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Drought and drought resistance

There are several definitions of drought which include precipitation, evapotranspiration, potential evapotranspiration, temperature, humidity and other factors individually or in combination[1].

However, for a farmer or a land area, drought is lack of available moisture (including that in the soil), which adversely affects crop productivity; it is also the failure of available water for irrigation to raise a crop. Since there is a loss in yield due to drought (or to put it more correctly, due to water-deficit stress), the drought resistance would be the mechanisms that will help in reducing or minimizing the loss. Drought has been defined as 'the inadequacy of water availability, including precipitation and soil moisture storage capacity, in quantity and distribution during the life cycle of the crop to restrict expression of its full genetic yield potential'[2]. The drought resistance is thus defined as 'the mechanism(s) causing minimum loss of yield in a water-deficit environment relative to the maximum yield in a water constraint free management of the crop'. We are, therefore, dealing with atleast two dynamic components. One is water availability through precipitation or otherwise with the uncertainties in time, distribution and amount, and the other is the plant itself going through all stages

of growth and development starting from seed germination to grain development. Matching these two dynamic processes in a manner that the influence of water deficit on yield is minimized becomes a matter of probability. This is unlike disease or pest resistance where a genetic relationship between the host and the parasite can be established and utilized through both conventional methods of plant breeding or molecular tools and genetic engineering with a higher degree of success.

The nature of drought

The drought-prone regions of India are given in Figure 1, which are mostly the semi-arid regions of the country. Punjab and Haryana, which are highly productive regions of the country, are semi-arid, but for irrigation systems would be much less productive. Thus agro-ecologically these regions are semi-arid but they are agro-productive because of irrigation. We normally define kharif (monsoon season) and rabi drought as follows:

Kharif (monsoon) drought

The kharif drought may be caused by one or more of the following situations: (i) The rains may start late relative to the long term average date of arrival. (ii) First few days of rains are not enough for establishment, although these may be enough for germination. (iii)

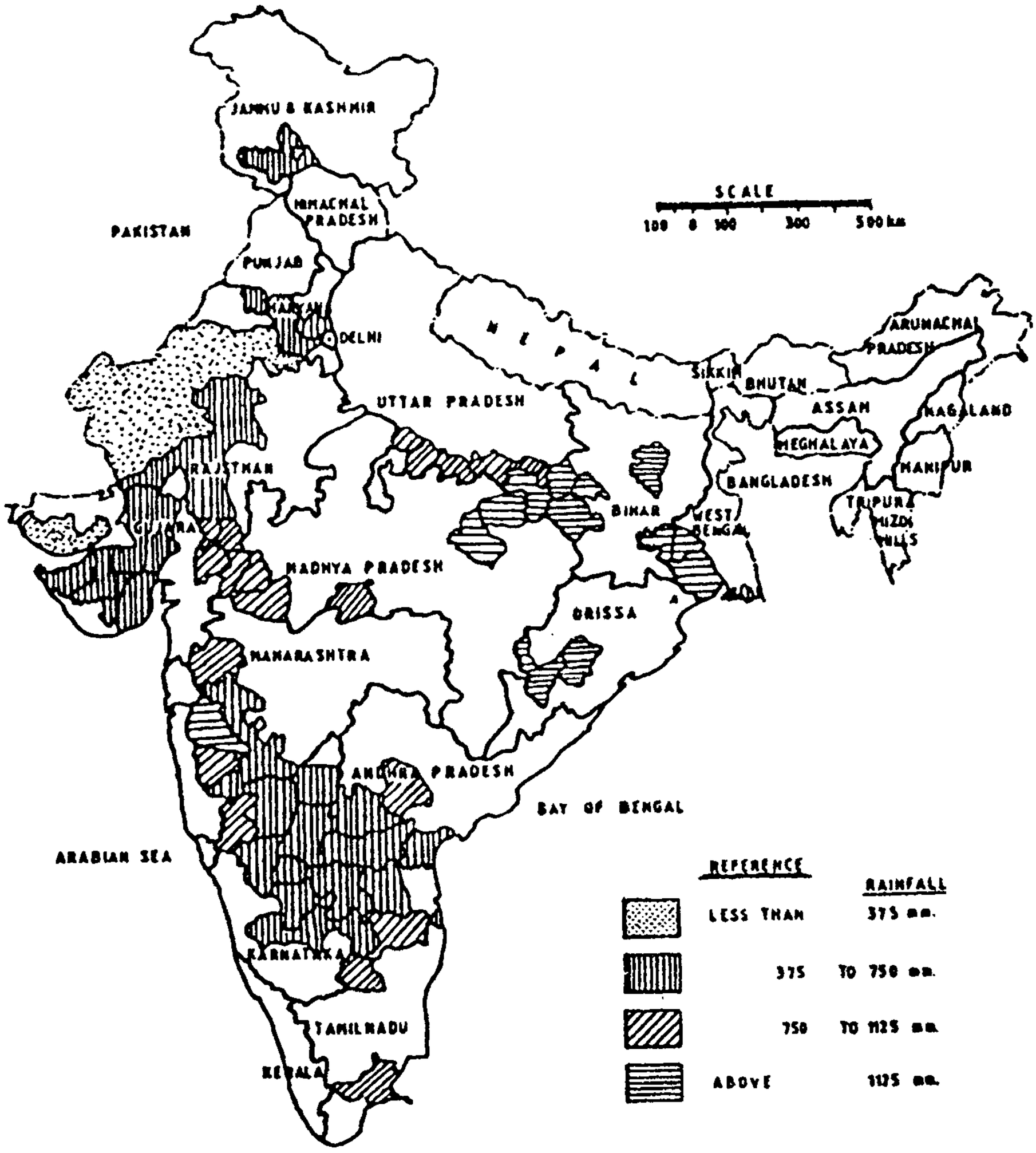

Figure 1. Drought-prone regions of India.

There may be breaks of more than a week in monsoon, and the maximum and minimum temperatures are 37°C, and 30°C respectively. (iv) There may be no uniform distribution of rainfall for example, most of the rain comes in first 4 to 6 weeks causing terminal drought or water deficit at grain filling period. (v) The total rainfall is less than normal (long term average), could be 19 to 60%.

Rabi drought

Rabi drought is relatively better predictable, and will be observed under the following situations: (i) When there is not enough soil moisture at the start of planting; (ii) When there are no winter rains; and (iii) When there is not enough moisture in soil profile in post-anthesis period.

It must be recognized that the capacity of the soil to store water, and the atmospheric temperature are important factors in determining the nature of drought. Since so many variables at each location differ from year to year, the performance of a crop may not be repeatable every year at the same place. Consequently, location specificity of cultivars in combination with stability for yield in grain crops become the desirable objective.

Crop requirements for productivity

The crop productivity depends on various phases from germination to grain development. The objective is to obtain yield through development of yield components which, in turn, depend firstly on mechanism of flower differentiation and secondly on a balance between growth and yield components. We can broadly classify the various phases as follows: (i) germination and establishment; (ii) vegetative growth; (iii) differentiation and flowering; (iv) development of yield components; (v) grain development.

Indeed a number of genes would be involved in each of the above five stages, and the question would be of their expression in response to various abiotic stresses. If there is no stress, crops would pass through these stages without any loss in productivity. However, one or more of these phases can be disturbed by any unexpected change in the environment. The following examples may be considered:

1. Germination and vegetative growth occurs but it is followed by a long spell of dry period. The plants must survive and in case the water becomes available there is resumption of growth.
2. Stress could come at the time of differentiation which may influence flowering or emergence of spike in cereals, or cause abscission of flowers.
3. The water deficit could occur at anthesis and during grain development affecting both the source as well as the sink, directly affecting the grain development.

Therefore, it is difficult to extrapolate the results of microorganisms to higher plants particularly if crop yield-based resistance to stresses is the objective since the level of organization is different. Nevertheless, we need to consider the development in characterization and isolation of genes attributed to drought stress.

Genes for resistance to drought stress

There has been substantial progress in identifying genes for resistance to various abiotic stresses such as temperature, salinity, waterlogging, nutrition, drought and others. This discussion will be confined to only a few transgenics overproducing osmolytes or stress proteins and tested for tolerance to drought stress (Table 1).

Proline

A strong correlation was reported between proline

Table 1. Transgenics produced for improved drought and salt tolerance

Transgenic over-expressing	Plant	Water relations	Claim	Ref.
Mannitol	Tobacco	None	Tolerance to salinity selectable	Tarczynski *et al.*[22]
Glycine-betaine	Tobacco chloroplast	None	Marker for osmoprotectant	Rathinasabapathi *et al.*[54]
Fructan	Tobacco	None	Resistance to drought stress	Pilon-Smits *et al.*[16]
Proline	Tobacco	None	Tolerance to osmotic stress	Kavi Kishore *et al.*[13]
LEA	Rice	None	Tolerance to water deficit and salinity	Xu *et al.*[30]

Mostly grown in vermiculite which has high water-holding capacity.

accumulation in seedlings of barley varieties and stability in yield under different drought conditions[3]. Yield stability was used as a measure of drought resistance and was based on yield at different locations which differed in precipitation[4]. This prompted search for genes associated with proline accumulation. However, the most stable variety Bankuti Korai was earliest in flowering and maturity, thus escaping drought. Within the drought-resistant variety Excelsior and the drought-susceptible variety Proctor (drought resistance and drought susceptibility based on stability) considerable seed to seed variation was found[5], thus questioning the earlier claims[3]. However, the proline-accumulating mutants in *E. coli* had survived at a much higher concentration of sodium chloride[6], which was erroneously equated to drought resistance. Proline was subsequently shown to be a compatible solute that will provide protection against denaturation of enzymes caused by high temperature[7]. Some genes related to proline synthesis, proline transport and accumulation have been identified[8–10]. P5C synthetase and proline dehydrogenase genes involved in the synthesis and degradation of proline have been cloned[11,12]. Salt stress and dehydration induce the expression of the gene for P5C synthetase and down-regulates the proline-dehydrogenase gene[9]. It was shown that transgenic tobacco plants overexpressing P5C synthetase gene exhibited high level of the enzyme and produced 10–18 fold more proline than control plants. These results suggest that activity of the first enzyme of the pathway is the rate limiting factor in proline synthesis[13]. The overproduction of proline enhanced root biomass and flower development in transgenic plants under drought stress conditions. However, the mechanism through which the increased cytoplasmic proline concentration may influence water relations at the whole plant level is not understood[14]. The enhanced root growth provides advantage in drought environments only if water is available at lower layers in the soil profile. There is no decline in solute potential of transgenic plants despite overexpression of solutes. These results are not explainable on the basis of current knowledge of water relations in plants.

Fructan

Fructans are polyfructose molecules that are produced in only 15% flowering plant species, including wheat and barley. It functions mainly as a storage carbohydrate but being soluble may help plants survive periods of osmotic stress induced by drought or cold, by varying the degree of polymerization of the fructan pool. The petal opening in daylily involves the conversion of fructans to low degree of polymerization products which help in osmotic adaptation[15]. To investigate the possible functional significance of fructans in drought stress, the bacterial gene *SacB* from *Bacillus subtilis* encoding levan sucrose was introduced into tobacco, a non fructan-accumulating plant[16]. The transgenic tobacco that produces bacterial fructans was produced and examined for growth performance under PEG-mediated drought stress. The growth of the transgenic plants was significantly higher both on fresh weight and dry weight basis under drought stress compared to the wild type tobacco. The transgenic tobacco exhibited significantly more biomass accumulation in roots under drought stress and higher non-structural carbohydrate content under all conditions. Hence we conclude that fructan synthesis in tobacco leads to enhanced resistance to drought stress. However, the mechanism of the improved performance is not clear since fructan concentration is too low to have an osmotic effect.

LEA

Osmotic stress induces the accumulation stress of a set of low-molecular weight proteins known as dress proteins in plant tissues such as LEAS and dehyrins. LEA proteins were first characterized in cotton as a set of proteins that are highly accumulated in the embryos at the late stage of seed development. Subsequently, many LEA proteins or their genes have been characterized from different plant species. LEA proteins were classified into three major groups based on their common amino acid sequence domains[17]. The group 3 LEA proteins have been implicated in stress tolerance based on the correlation between LEA gene expression and accumulation of LEA proteins and tissue dehydration tolerance in dehydrated wheat seedlings[18]. In rice seedlings, the levels of group 2 LEA proteins and group 3 LEA proteins were higher in roots and induced by ABA and salt in salt-tolerant varieties compared to salt-sensitive varieties[19]. However, the functions of LEA protein and their physiological role are not known. The transgenic approach was adopted to investigate the function of the HVA 1 protein in stress protection[20]. They produced rice transgenics carrying HVA 1, a group 3 LEA protein which expresses in barley aleurone and embryo during late seed development correlating with the seed desiccation stage[21]. The transgenic rice plants exhibited constitutive high expression of HVA 1 protein ranging from 0.3% to 2.5% and 0.3% to 1.0% of the total soluble protein in leaf and root respectively. The R1 progeny of three transgenic plants was used for evaluation of the growth performance under water deficit and salt stress treatment. The appearance and development of the major damage symptoms such as wilting, dying of old leaves and necrosis of young leaves caused by the stress conditions were delayed in the transgenic plants. On removal of stress treatments, the transgenic plants showed better recovery than did control plants. The better performance of R1 transgenic lines under stress conditions was correlated with higher level of HVA 1

protein accumulation in their RO plants. The mechanism whereby LEA protein accumulation provided stress tolerance has not been examined. In fact several cycles of drought stress treatment have been given before recording growth data which implies acclimation response.

Mannitol

Plants produce and accumulate osmotically active low-molecular weight compounds such as sugar, alcohols, proline and glycine betaine. Osmolytes may contribute towards osmotic adjustment besides providing protection to macromolecules such as enzymes and proteins from electrolytes and temperature. Transgenic tobacco plants were produced that synthesized and accumulated the sugar alcohol mannitol by introducing a bacterial gene *mtL* that encodes mannitol 1-phosphate dehydrogenase[22]. The mannitol overproducing tobacco exhibited an increased ability to tolerate high salinity in terms of maintenance of leaf and root growth. The mechanism whereby mannitol could provide salt tolerance is not known. Mannitol-containing seeds produced from transgenic *Arabidopsis thaliana* overproducing mannitol exhibited enhanced germination under high salinity environment[23]. The mannitol present in the seeds was probably acting as a compatible solute. However, more work is needed to investigate the mechanism involved.

Water stress proteins and heat shock proteins have also been identified in response to water stress and heat shock in higher plants[24–26]. Though the function of some of the genes encoding these proteins is being investigated, it is difficult immediately to predict whether the presence of these genes could impart resistance to water deficit and high temperature.

The limitations in the studies on response of transgenics overproducing osmolytes or stress proteins to water deficit or salt stress are: i) The drought stress has not been quantified either as soil water deficit or in terms of plant water relations. *ii*) It is known that plant response to stress varies at the vegetative and the reproductive stage. The results obtained with vegetative plants may not be comparable to those obtained during seed development. Tobacco is not the best example of reproductive stage because it partitions only a small amount of current/reserve assimilates for seed development unlike grain crops. The drought score changes depending upon the grain yield capacity. The transgenics have been examined for drought and salt tolerance mostly during vegetative stage. More research is needed on the transgenics under various stress intensities at various growth stages.

Molecular marker technology and drought resistance

The development of molecular markers for use as probes for genomic DNA has revolutionized the genetic analysis of crop plants and provided the geneticists, physiologists, agronomists and breeders with valuable new tools to identify traits of importance in improving resistance to abiotic stresses. The availability of a genetic map saturated with molecular markers allows a breeder to do selection for certain characters much more efficiently and effectively than was possible previously. Physiologists have identified a few traits that should be beneficial in improving drought responses, such as osmotic adjustment, water use efficiency and an efficient root system. Quantitative trait loci (QTL) for root characteristics have been identified in rice[27] and maize[28]. Osmotic adjustment helps to maintain shoot functioning[29] and increased capacities for osmotic adjustment have been reported to improve yields in droughted plants[30] though not always[31,32]. QTL analysis of osmotic adjustment in rice has been carried out recently.

In maize anthesis-silking interval (ASI) has been identified to be a major determinant of yield under drought conditions[33]. QTLs have been identified for the reduction of ASI under drought in maize[34] and it is argued that MAS based on ASI QTLs should be a powerful tool for improving drought tolerance of maize inbred lines. In order to improve yield under drought in common bean, a study was initiated[35] (i) to identify RAPD markers for yield performance both under moisture stress and nonstress conditions, (ii) to compare the MAS to conventional phenotypic selection for drought resistance and to determine if the identified markers are useful in population other than the one from which the markers were identified. The study was conducted with two recombinant inbred populations across eight locations under stress and nonstress conditions. The results indicated that RAPD markers were population specific. The MAS improved yield performance marginally under drought in one population where the conventional selection failed. In another population, the conventional selection was three times more superior than MAS. Hence there are several limitations in this approach and more studies are needed to show the effectiveness of MAS to improve yield under drought environments.

Physiological analysis of yield under drought stress

The yield of a plant and a crop depends on the development of yield components (sink) and the availability of assimilates including carbohydrates, proteins, minerals, etc. A complementary relationship among them is essential for obtaining yield[36]. If the source is affected, when the 'sink' is being established, the size of the 'sink' becomes poor, leading to low yield. Also, there is a close relation between the available water in soil profile in post-anthesis period and yield[37,38].

There is a change in score in response to water deficit stress. This phenomenon was studied using male sterile and fertile lines of wheat and sorghum in pots and the field respectively[39]. With the decreasing available water and increasing water stress in the vegetative stage, there was a decline in leaf area and chlorophyll content (Figure 2). There was no difference between sterile and fertile lines. However, when the same lines were water stressed during the post-anthesis period, the fertile lines showed a sharper decrease in leaf area and chlorophyll content (Figure 3). Further studies in mung bean and cowpeas showed that the plants stressed in vegetative stage are capable of recovery after irrigation, when recovery is measured in terms of various traits such as water potential, photosynthesis rate, leaf area and chlorophyll content[40–42]. However, the same plant does not recover if the stress is experienced during pod development (Figures 4, 5 and 6). Thus drought-induced senescence, which is often seen in vegetative stage, is accentuated and accelerated in a plant undergoing seed development[43]. Why does such a change occur? Does it imply that any selection for drought or abiotic stresses in vegetative (seedlings/plantlets in tissue culture) stage will not express during grain development if the plant experiences stress at that stage? How are the transgenic plants with overexpression of proline, mannitol synthesis and others likely to respond? It is the actual experimentation alone that will provide the answer to these questions, but we can consider the sequence of events which occur during the life cycle of a crop plant.

The vegetative phase establishes the plant by investing in root system, leaves and stem. They accumulate substrates such as carbohydrates and proteins. Greater the investment in root system, greater is the possibility of

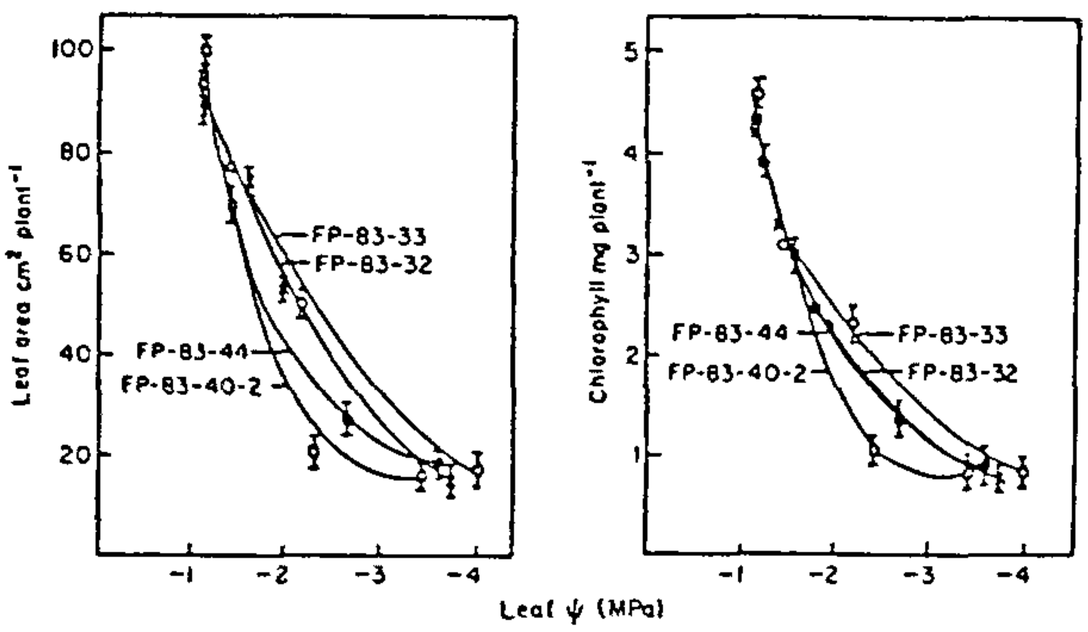

Figure 2. Effect of water stress on leaf area and chlorophyll content per plant in male fertile lines FP-83-44 (o—o) FP-83-33 (•—•), male sterile lines FP-83-40-2 (×—×) and FP-83-32 (□—□) of wheat at the seedling stage. Values ± SE for leaf Ψ were as follows: FP-83-44:1.2 ± 0.06; 1.7 ± 0.09; 2.6 ± 0-1 and 3.5 ± 0.2 MPa, respectively. FP-83-40- 2:1.1 ± 0.05; 1.5 ± 0.03; 2.3 ± 0.07 and 3.4 ± 0.05 MPa, respectively. FP-83-33: 1.15 ± 0.05; 1.4 ± 0.1; 2.2 ± 0.1; 4.0 ± 0.2 MPa, respectively. FP-83-32: 1.15 ± 0.05; 1.6 ± 0.3; 2.0 ± 0.2 and 3.7 ± 0.4 MPa, respectively.

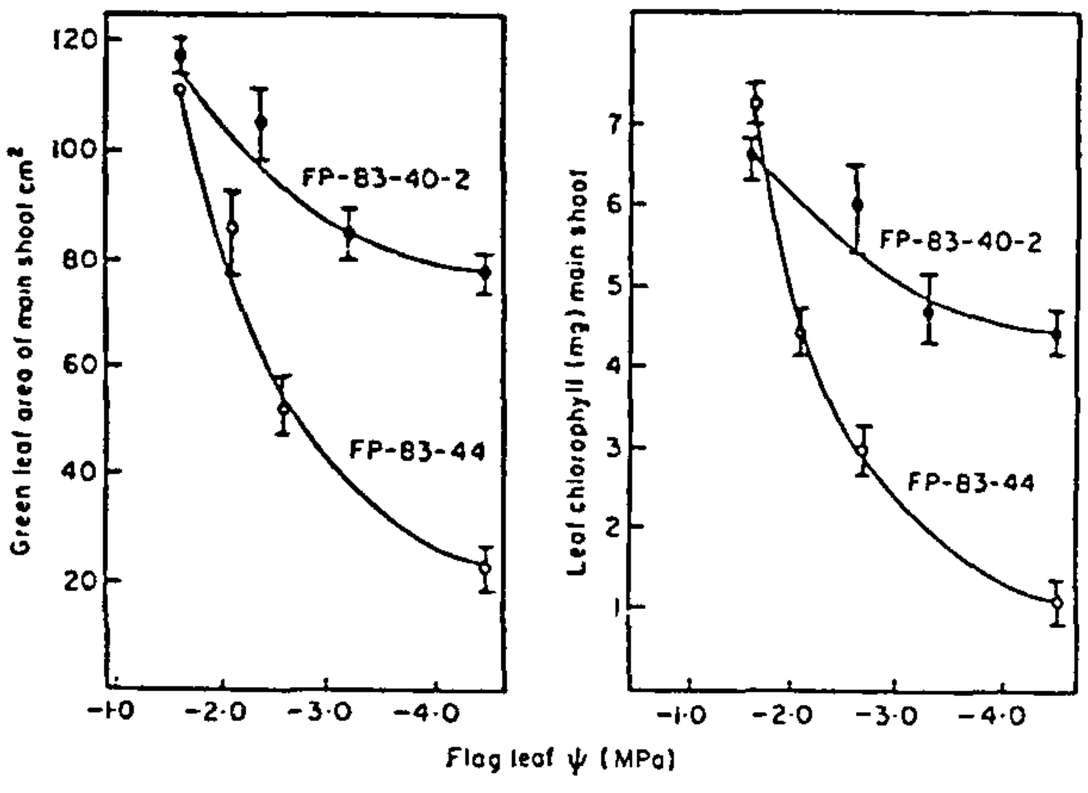

Figure 3. Effect of water stress on leaf area and chlorophyll content of main shoot in male fertile line FP-83-44 (o—o) and male sterile line FP-83-40-2 (•—•) of wheat during grain development. Values ± SE of leaf Ψ were as follows: FP-83-44: 1.6 ± 0.2; 2.1 ± 0.2; 2.6 ± 0.3; 4.5 ± 0.5 MPa, respectively. FP-83-40-2: 1.6 ± 0.2; 2.35 ± 0.2; 3.2 ± 0.3 and 4.5 ± 0.4 MPa, respectively.

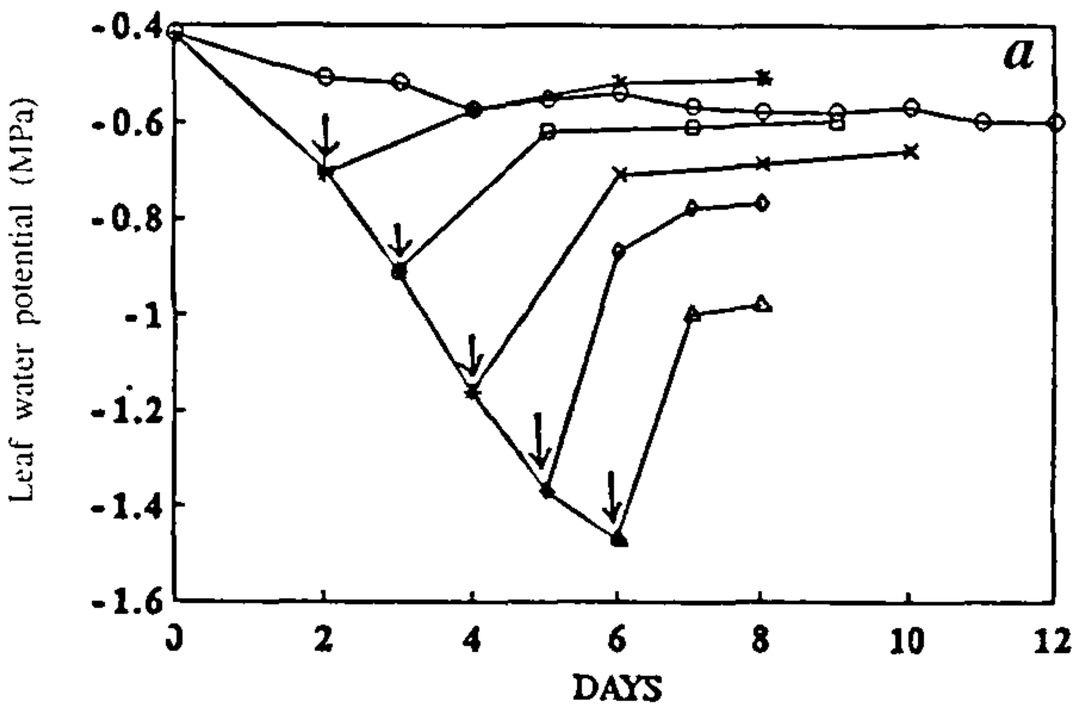

Figure 4. Effect of water stress and subsequent rewatering (↓) during vegetative (*a*) and reproductive stage (*b*) on leaf water potential in cowpea. C (o—o), S (2D *, 3D □, 4D ×, 5D ▱, 6D Δ).

exploring water and nutrients if the soil depth is adequate. The vegetative stage is followed by reproductive stage through flowering and seed set. This influences the intraplant competition and the development of seeds (reproductive sink) is preferred at the cost of other plant parts depending upon the nature of the plant – a dicot indeterminate (pulses and oilseed), a dicot determinate, a cereal with or without tillering. This competition usually results in decreased uptake of nutrients and mobilization of nitrogen and carbohydrates from leaves and stem to seed development. It is one of the major factors because of which male sterile plants show delayed senescence. However, the aerial parts committed to senescence possibly mobilize and transport reserves to roots. In perennial plants, this would help in survival. In plants developing seed, the supply of reduced nitrogen and carbohydrates is thus crucial. If nitrogen from chloroplasts (Rubisco) is mobilized, this would hasten senescence and hence the drought score. Would the plants having overproduction of proline or mannitol meet this requirement, particularly in case nitrate reductase activity is reduced[44] and synthesis of reduced nitrogen declines? It would be more realistic to presume that change in one trait may not overcome stress effects and intraplant competition together leading a plant to be productive in respect of grains/seeds. In fact we need to distinguish clearly the metabolic functions at cellular level, growth, differentiation and yield. Recent studies on transgenic plants having overexpression of proline describe these chemicals as osmoprotectants, though it hardly contributes to osmotic potential in crop plants[45,46]. Therefore, the term becomes untenable if not misleading. Proline and glycine-betaine have been referred to as compatible solutes, thereby suggesting that accumulation of these molecules is compatible to other cellular functions unlike being detrimental or toxic both during stress as well as in the recovery from stress[7]. Therefore, it is possible that accumulation of proline in higher plants may help in maintenance or in recovery of cellular functions, particularly of some enzymes such as malate dehydrogenase, and others. However, it would be unrealistic to relate it to grain yield in a crop, though it might be important in survival during stress and recovery on alleviation of stress. This could be assessed from

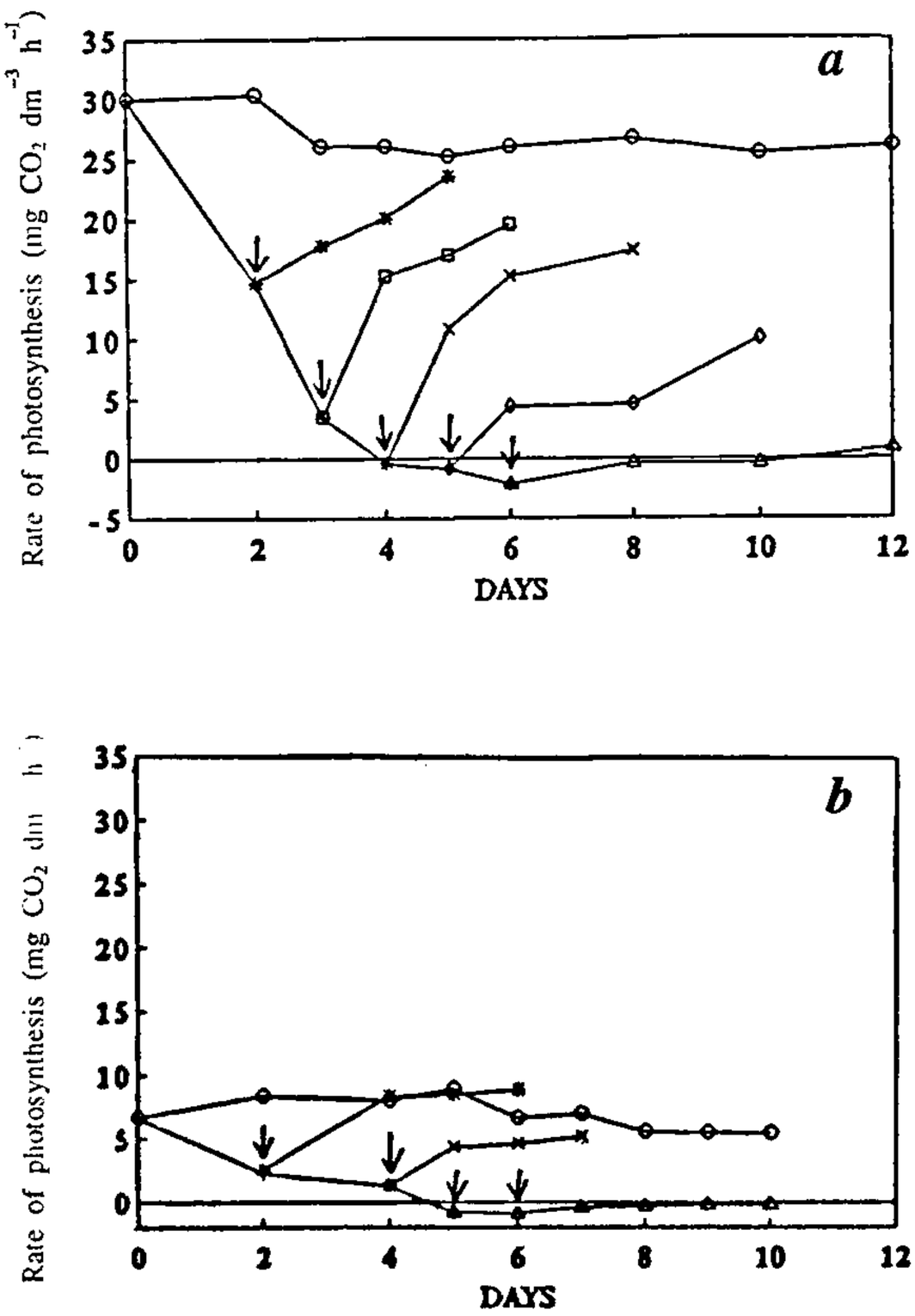

Figure 5. Effect of water stress and subsequent rewatering (↓) during vegetative (*a*) and reproductive stage (*b*) on photosynthesis rate in cowpea. Symbols are the same as in Figure 4.

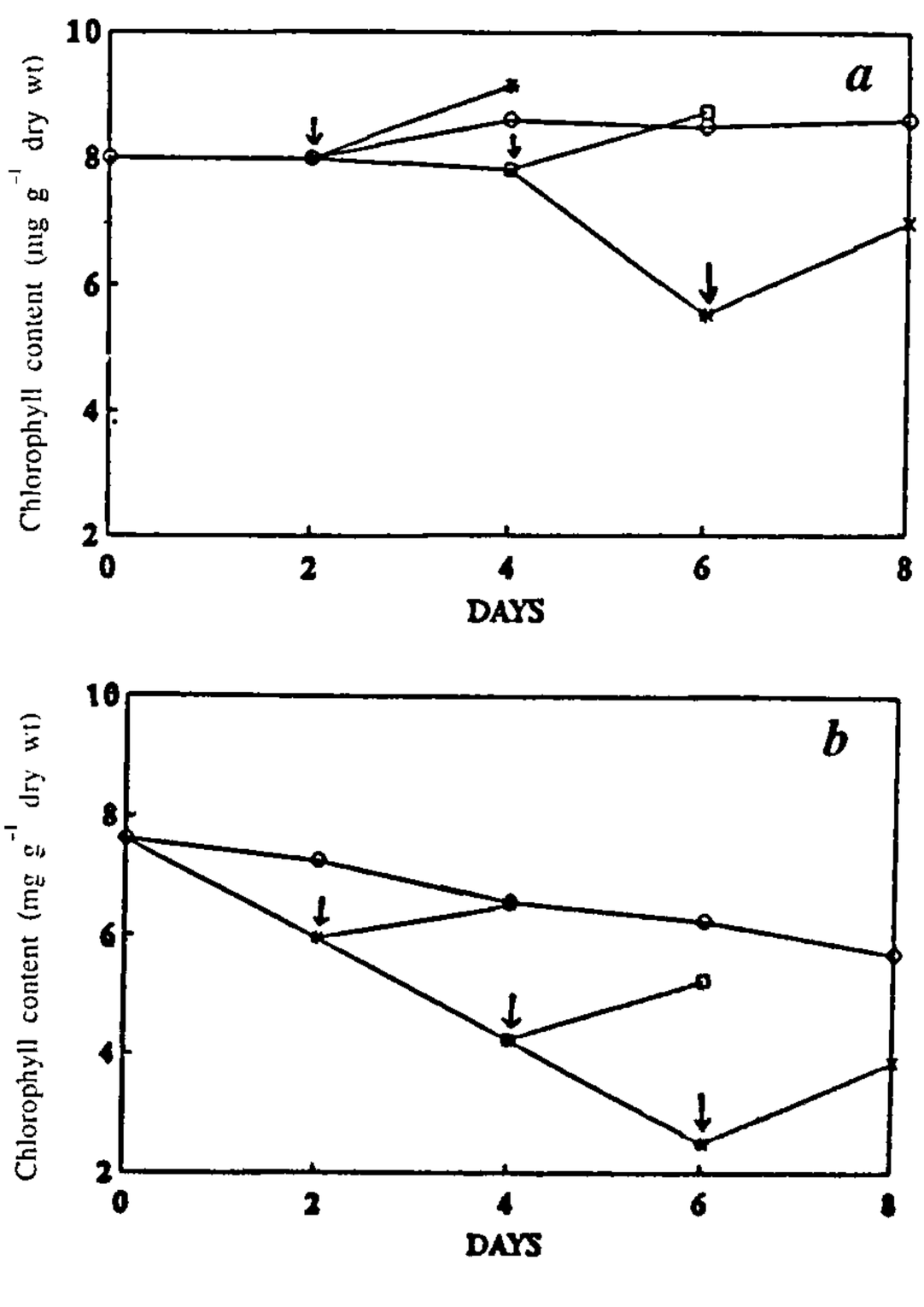

Figure 6. Effect of water stress and subsequent rewatering (↓) during vegetative (*a*) and reproductive stage (*b*) on chlorophyll content in cowpea.

the data on the number of flowers and seeds in transgenic tobacco which do not partition a substantial amount of substrates or reserves from leaves and stem to seed production. The latter have a small demand because of their size and weight. Interestingly[13] no data on dry matter or seed weight was given for transgenic tobacco overproducing proline. Therefore, it is difficult to evaluate such results. It is, therefore, reasonable to infer that the model systems such as tobacco are good for assessing the importance of some traits such as insect or pest-resistance, but not for such responses which involve intraplant competition at different stages of growth, development and grain development.

Alternative approach

Biotechnology is a powerful tool and in the long run will have a strong impact on agriculture including grain crops. The present status of biotechnology in the areas such as stress resistance is rather modest. Therefore, it is difficult to foresee abiotic stress-resistant transgeneics in the next five to ten years growing in farmers' fields anywhere in the world. This is particularly true of drought-resistant and temperature-resistant crops. And yet the population of the developing countries including India would grow, and with that the demand for food grains would grow. Therefore, some of the simple field-based approaches might help in the near future.

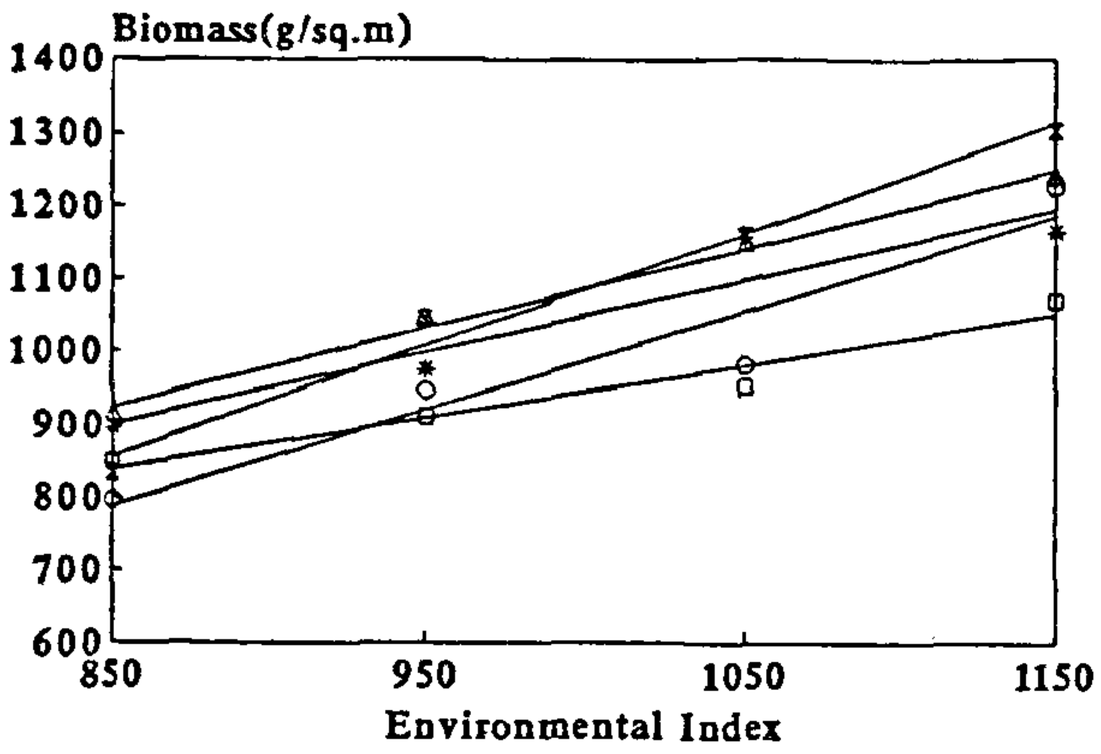

Figure 7. Biomass vs environmental index in wheat varieties and rainfed selections. o, C306; Δ, Kundan; ∇, HD2329; □, RS635 and *, RS640.

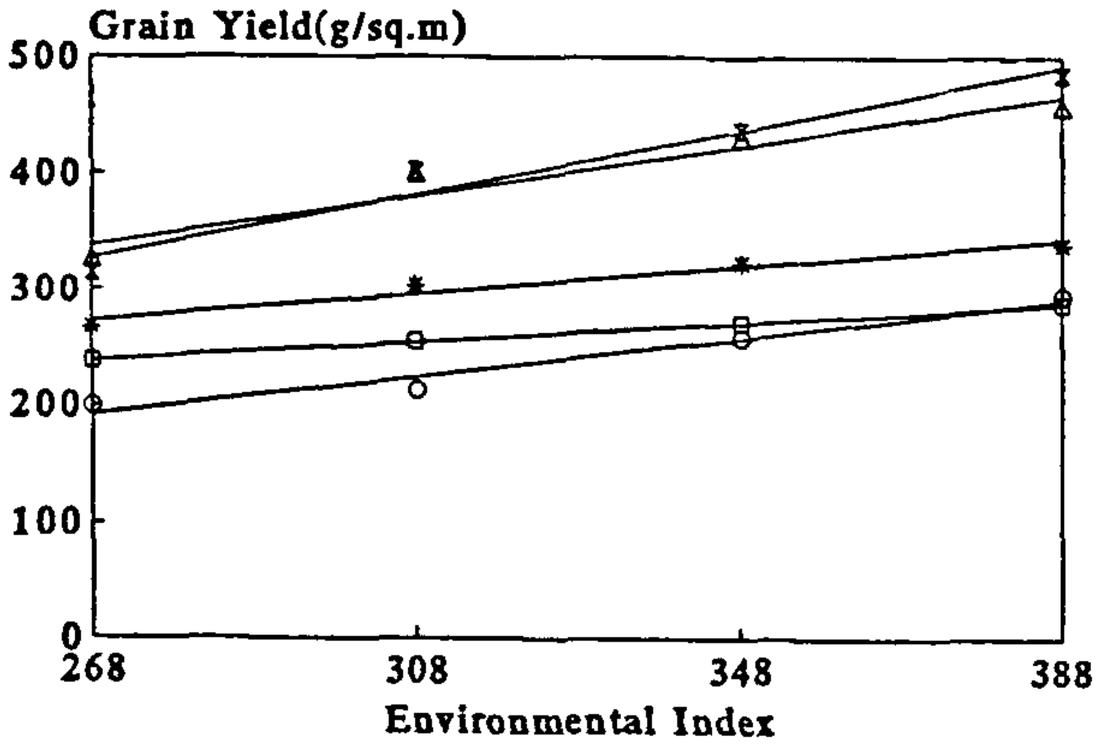

Figure 8. Grain yield vs environmental index in wheat varieties and rainfed selections. Symbols are the same as in Figure 7.

Delayed flag leaf senescence in wheat

Several studies in the past have shown that the flag leaf in wheat is a major contributor to grain development[47]. Therefore, the selection proceeded to identify genotypes which had almost erect flag leaf enabling photosynthesis for a longer duration[48,49]. Similar efforts succeeded in rice. Consequently, the modern varieties of rice have medium erect flag leaf emerging out of the crop canopy. However, in rainfed conditions no such effort was made. Furthermore, as the grain development commences, there is in fact decline in flag leaf area coupled with leaf senescence[50,51]. There are a larger number of genotypes evaluated every year in All-India coordinated trials but there is usually no evaluation of individual traits except year and grain weight.

A few genotypes having delayed flag leaf senescence were selected in 1991–92, and multiplied for evaluation in a range of water availability created through the line-source irrigation system[52]. The results of two seasons of grain yield and biomass accumulation at four levels of water availability are given in Figures 7, 8 and Table 2. It appears that a trait such as delayed senescence of the flag leaf could be advantageous under field conditions. However, we do not know much about the genetics of flag leaf size or delayed senescence. We also do not know much about the stability of these traits. This is only one instance but eventually we need to split yield into different morphological components. For example, the importance of seed germination at different levels of soil moisture (soil water potential or matrix potential) needs to be associated with tillering or branching and vegetative biomass. Do these traits have a genetic control,

Table 2. Stability index of grain yield and biomass production of wheat cultivars and rainfed selections in drought stress environments

Variety	Yield	Biomass
C306	0.90	1.12
Kundan	1.46	0.77
HD2329	2.25	1.36
RS635	–0.04	1.04
RS640	0.61	1.15

and what is their stability and genetic variability? The assimilate availability at the time of differentiation (spike differentiation and development of flower and bud) is essential for the development of yield components. Subsequently, the stability of flower buds (including spikelets) and the fertility of pollen becomes necessary. This is followed by a complementary relationship between 'source' and 'reproductive sink' to achieve a higher level of productivity as in heterotic hybrids[36]. This amounts to saying that there is a need to put together building blocks to achieve the objectives of yield in an environment of abiotic stresses. The processes such as osmoregulation are important but under field conditions are not stable[53]. This is because there is no stability of environment in different crop seasons. It would thus be important to establish relationship between the stability of processes and the stability of morphological traits contributing to yield components and yield.

Limitations of the present approaches of plant breeding

There are some general perceptions of drought-resistant genotypes, though often such genotypes are low or moderate in yield. There is hardly any basis of selection in F2 or F3, but subsequently some lines are identified which appear promising. They are tested at different locations. This has been done more specifically in wheat. The evaluations of rice cultures in upland represent selection for drought tolerance. There are no such directed efforts in pulses, oilseeds and coarse cereals. In any case, the environment is not defined in terms of rainfall, soil moisture, temperature and vapour pressure deficit. Consequently, the performance of the same genotype varies year after year. Since drought and temperature alone and in combination lead to location specificity, it is unlikely to obtain genotypes with wide-adaptability as observed in irrigated environments. Therefore, any effort to average out the results of trials of a region may not be the best evaluation. However, it is possible that a wider adaptability for phenology with adjustment to environmental responses may be achievable. The check itself varies year after year. Therefore, appropriate models could be used to predict the maximum yield potential at a location in a given year. This should serve as a reference for comparing the varieties.

Conclusions

Biotechnology has the potential for being effective in improving crops for various traits. However, the dynamic nature of environment as well as plants imposes limitations for achieving drought and temperature resistance through biotechnology in field crops. This may not happen even in a 5–10 year period. In the meantime, identification of traits for different phenological stages and those contributing to yield, need to be pursued for pyramiding traits through conventional means.

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MEETINGS/SYMPOSIA/SEMINARS

National Symposium on AIDS

Date: 26–28 February 1998
Place: Dehra Dun

Objectives of the symposium: To bring mass awareness among general public, especially vulnerable groups, about HIV/AIDS; To provide better medical and health care facilities for AIDS patients; To counsel HIV-affected patients; To exploit media for AIDS education; To train human resources for the development of foolproof diagnosis and treatment of AIDS; To promote research for drug development and non-institutional care for AIDS patients; To develop a system for narrowing the gap between government and private agencies involved in prevention and control of AIDS.

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Diversity of Social Insects and other Arthropods and the Functioning of Ecosystems

Date: 7–9 March 1998
Place: Mudigere

The congress is intended to discuss the following topics: Measuring and monitoring insect diversity; conceptual and empirical issues; Diversity and community structure of arthropods – social insects as a component of insect diversity; Role of social insects in ecosystem functioning (as pollinators, predators, scavengers, soil-builders etc.); Social insects and productivity of terrestrial ecosystems; Evolutionary ecology of social behaviour in relation to ecological diversity; Anthropogenic pressures on arthropod diversity and functioning of ecosystems; Conservation of social insects and other arthropods: assessing status and developing strategies. A discussion meeting on 'Development and linkages of databases on insect diversity' will also be held. Abstracts in Current Science format not exceeding 300 words could be submitted in type or electronic form to the organisers at Mudigere before 30 January 1998.

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Potential applications of antisense RNA technology in plants

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The antisense RNA technology involves the cloning of a particular gene in reverse orientation with respect to the promoter, such that the coding strand acts as template strand and vice versa. The antisense gene when transcribed gives rise to RNA which is complementary to sense mRNA, thus inhibiting the target gene expression by forming RNA duplex which is unstable. Besides, antisense oligonucleotides can also be administered exogenously to manipulate the expression of a particular gene. The concept of involvement of antisense RNA in gene regulation in natural systems is not new, however, recently only the antisense strategies have been used both in plant and animal systems for ascertaining gene function, manipulation of gene expression and in therapies. Though many potential applications of this technology have been put forward, several questions still remain unanswered, especially the stability and mechanism of action of antisense RNA and the relationship between the levels of inhibition and concentration of antisense RNA. The current status, including potential applications of this emerging field in plants is briefly reviewed here.

THE study of genetics for identifying a gene and its function traditionally involves the use of naturally occurring or experimentally induced mutants. This has certain disadvantages as any gene may be mutated and identification of a mutant gene can be tedious. Besides, it cannot be ascertained whether the mutant phenotype is the result of mutation in a particular gene. This particular disadvantage has been overcome by the technique of site-directed mutagenesis, yet in cases where mutation in a particular gene is lethal or a particular gene is present in multiple copies, the generation of mutants is futile.

In cases where a gene has been identified and assigned a particular phenotype, additional approaches are often required to exactly probe the function of gene.

Such hurdles in gene identification and manipulation can be overcome by antisense RNA technology. It involves the cloning of a gene in reverse orientation with respect to the promoter such that the coding strand acts as a template and the sequence of mRNA is the same as the opposite strand or the coding 'sense' strand. The gene cloned in reverse orientation or the antisense gene when transcribed gives rise to mRNA having the sequence complementary to the sense mRNA. The RNA–RNA binding of the sense–antisense RNA strands leads to inhibition of sense mRNA expression (Figure 1). Besides antisense RNA, sequence complementary to sense mRNA can also be administered exogenously to be able to manipulate gene expression. The antisense oligonucleotides bind to complementary mRNA and prevent its transport to cytoplasm or translation into protein. DNA–RNA hybrid serves as substrate for ribonuclease RNAase-H which specifically degrades RNA strand in a DNA–RNA hybrid.

The first effort that definitely demonstrated the blockage in translation due to the use of antisense RNA in cell-free extracts (CFEs) was carried out by Singer *et al.*[1]. They showed that synthesis of polyphenylalanine in CFE with polyuridylic acid as template was completely inhibited when polyadenylic acid was added to the translation mixture.

This article briefly describes the basic and applied aspects of antisense RNA technology, particularly in plant systems.

Natural antisense RNA regulation of gene expression

Naturally occurring antisense RNA was involved in gene

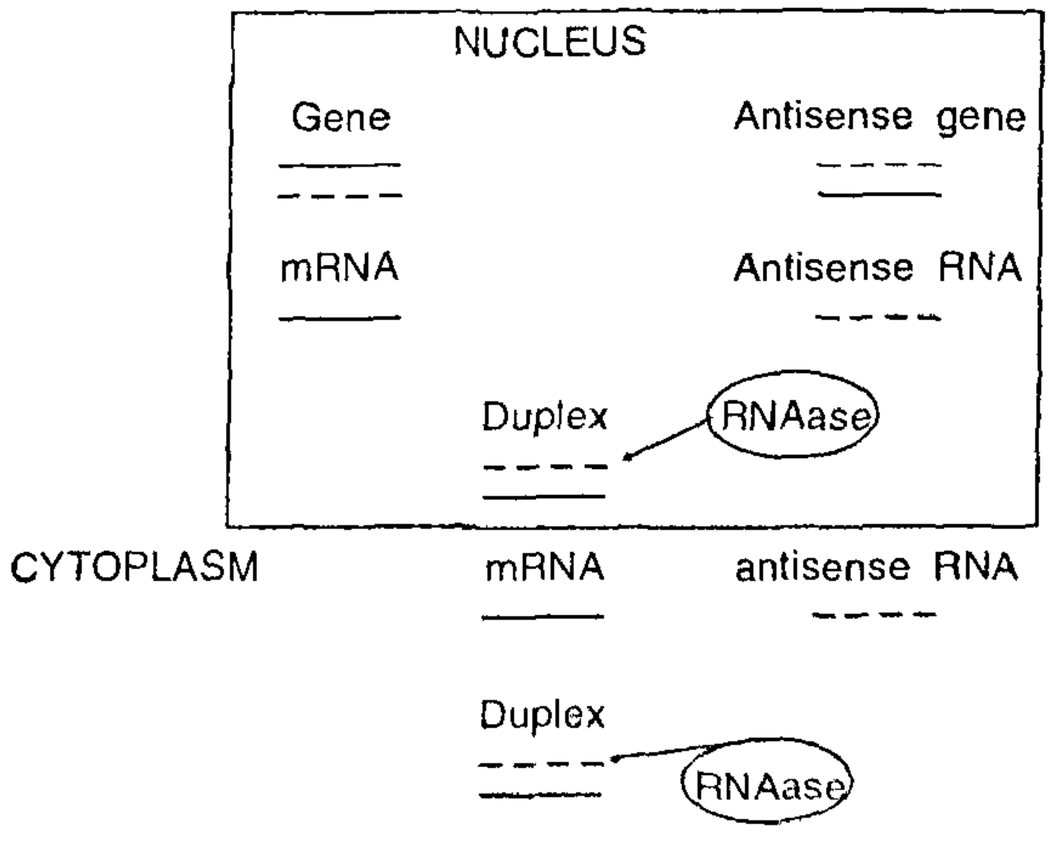

Figure 1. Proposed mechanisms of action of antisense RNA.

*For correspondence.