Recent developments in transgenics for abiotic stress in legumes of the semi-arid tropics

Kiran K. Sharma and M. Lavanya

Genetic Transformation Laboratory
International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)
Patancheru, Andhra Pradesh 502 324, India.

Abstract

The semi-arid tropics (SAT) are characterized by unpredictable weather, limited and erratic rainfall and nutrient poor soils and suffer from a host of agricultural constraints. Several biotic and abiotic stresses affect crop productivity. Mandated crops of ICRISAT that include groundnut, pigeonpea, chickpea, sorghum, and pearl millet are the main staple foods for nearly one billion people in the SAT. Judicious application of biotechnological tools holds great potential in alleviating some of the major constraints to productivity of these crops. Gains in crop productivity through research advances in genetic enhancement will help to achieve sustainable food security, poverty alleviation, and environmental protection in the SAT. ICRISAT has a commitment to transfer the benefits of plant biotechnology to the developing world, as genes relevant to their crops and environment become available. Genetic transformation provides a complimentary means for the genetic betterment of the genome of these crops. Therefore, the research in transgenic crops offers a new means to achieve ICRISAT's mission, i.e., applying science to improve agriculture in areas of the world where sub-optimal rainfall and biotic stresses are the major constraints for crop productivity. In this pursuit, efficient protocols for the genetic transformation of the important legumes of the SAT including groundnut, pigeonpea and chickpea have recently been developed at ICRISAT. More recently, in collaboration with our partners, we have initiated work on the development of transgenic plants for major abiotic stresses that include drought, salinity and low temperatures. Several gene transfer approaches have been shown to improve the stress tolerance of crop plants. The transferred genes include those encoding for enzymes required for the biosynthesis of various osmoprotectants, or those encoding enzymes for modifying membrane lipids, LEA proteins, detoxification enzyme, and stress-inducible transcription factors have been demonstrated to have great potential. This paper reviews the current status of transformation technology for the genetic improvement of legumes of the SAT and it's possible application for developing transgenic plants with enhanced tolerance/resistance to abiotic stresses.

Keywords: Abiotic stress; chickpea; drought; groundnut; pigeonpea; semi-arid tropics; transgenic plants

Introduction

Abiotic stresses like drought, low temperature, and high salinity are environmental factors that dramatically limit plant growth and crop productivity (Boyer, 1982). It has been estimated that 10% of the arable land can be classified under the non-stress category, which implies that the crops grown under the 90% of arable land experience different environmental stresses, singly or in combination. The prediction is that the water deficits will continue to be the major single abiotic factor likely to affect crop yields globally.

The semi-arid tropics (SAT) are prone to hot summers and erratic rainfall having the season length of less than 100 days (Virmani and Singh, 1986). Agriculture in these environments means growing marginal crops on marginal lands with marginal resources. The productivity of crops in these regions is severely affected by drought besides several biotic constraints (Sharma and Ortiz, 2000). Some of the most important crops of this region include groundnut, chickpea, pigeonpea, sorghum, and pearl millet that are the mandated crops of ICRISAT and are the main staple food for nearly one billion people in the SAT. Amongst

these groundnut, chickpea and pigeonpea are legumes that are significant groups of agriculturally important crops and have been the subject of widespread efforts to improve desirable traits. Though, quantitative estimates of yield losses due to drought in tropics are scarce and imprecise, according to some predictions (Subbarao et al., 1995), the productivity could be increased over the present levels in groundnut, chickpea and pigeonpea by 29%, 49%, and 57% respectively, if water was not limiting. However, in rainfed agriculture, there seem to be very few options to increase the area under irrigation and the only option is to improve the realizable yields under water deficit conditions. Although drought management has been an option to increase realizable yields, it is now increasingly being realized that the genetic improvement of drought tolerance is more rewarding (Udaya Kumar et al., 1998; Saxena, 2001). The seed-based technology seems to be easier to transfer to farmers than the more complex knowledge-based agronomic practices. As irrigation water sources become more scarce, development of crop cultivars with improved adaptation to drought is a major goal in many crop breeding programmes. Although, in the last two decades very encouraging progress has been made in assessing the stress responses of several plants (Blum, 1985; Boyer, 1996; Ludlow, 1993; Edmeades et al., 1996), the progress in developing genotypes with enhanced tolerance to abiotic stress has been very slow. Due to the complexity in the physiological, biochemical, and genetic traits involved in drought tolerance, an imperfect understanding of nature of drought, and using yield as an empirical selection criteria, no single strategy may suffice to develop stress tolerant genotypes. It has been recognized that genetic improvement is one of the major components of an integrated approach to stabilizing and improving crop production in drought environments (Subbarao et al., 1995). New tool of biotechnology offer impressive options to supplement the ongoing efforts on developing genetically enhanced germplasm for achieving sustainable food production in the SAT. Drought tolerance breeding might be facilitated by marker-assisted selection in the near future based on the development of molecular linkage maps for crop species (Ribaut et al., 1996, 1997; Nguyen et al., 1997; Zhang et al., 1999; Forster et al., 2000). In addition to classical and molecular breeding approaches (see Fig. 1), genetic transformation to introduce novel genes into plants for better tolerance to water deficit offers an attractive option (Tarczynski et al., 1993; Pilon-Smits et al., 1995; Xu et al., 1996; Sivamani et al., 2000). In fact, the genetic engineering approaches have been shown to be relatively fast and precise means of achieving improved stress tolerance (Hanson and Burnet, 1994; Bohnert and Jensen, 1996; Bohnert and Shen, 1999; Kasuga et al., 1999).
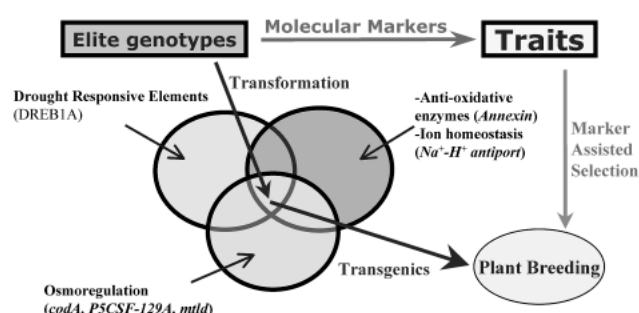

Fig. 1. A schematic representation of an integrated approach employing biotechnological tools and traditional plant breeding to develop abiotic stress tolerant crops.

Drought as a major abiotic constraint to productivity of legumes in the SAT

Drought though a broad term can be defined as a meteorological event which implies the absence of rainfall for a period of time long enough to cause moisture depletion in soil and water deficit with a decrease of water potential in plant tissues (Kramer, 1980). It prevents the crops from reaching the determined and expected yield and drastically lowers the production thus affecting the economy of a country. In an agronomic sense, drought stress has been defined as a reduction in grain yield attributable to plant water deficit (Subbarao et al., 1995). Further, the grain legumes dependent on current rainfall are prone to intermittent drought stress during the vegetative or reproductive growth period; the crop recovery from the drought is determined by subsequent rainfall. Terminal drought stress, which occurs during the pod-filling phase of crops, is common and a major yield reducer for crops growing with current rainfall (Nageshwara Rao et al., 1985a,b). This is even more critical for crops grown during a post-rainy season and reliant on stored soil moisture. Groundnut, chickpea, and pigeonpea that are major crops in SAT are very sensitive to high temperatures and water deficits during the flowering stage and seed/pod development stage resulting in heavy loss to productivity.

Groundnut. Groundnut (*Arachis hypogaea* L.) is a major oilseed legume native to south America and forms an important legume in Asia and Africa. It is mainly grown under rainfed, irrigated, and residual moisture conditions. The crop is cultivated on 24.8 million hectares with an average productivity of 1.32 t^{ha}. About 93.8% of the world's production of groundnut is grown by resource-poor, small farmers in 96.9% of world's groundnut area in developing countries where scanty and unseasonal and unpredicatable rainfall is

observed (Nageshwara Rao and Nigam, 2001). Asia accounts for 66.5% groundnut production on 56.8% area while Africa produces 24.7% on 38% area subjected to groundnut production. This also accounts for low average yields of 0.86 t ha^{-1} in Africa and 1.55 t^{-ha} in Asia as opposed to 2.5 t^{-ha} in developed countries despite the potential to produce 10 t^{-ha}. Poor yields in these countries is mainly due to the biotic and abiotic constraints like erratic rainfall, low residual moisture, lack of high yielding adapted cultivars, damage by pests and diseases, poor agronomic practices, and limited use of inputs (Nageshwara Rao and Nigam, 2001). In the event of end-of-season drought, which is common under rainfed and residual moisture conditions, the produce becomes vulnerable to aflatoxin contamination. The loss due to biotic factors that include various diseases caused by fungal pathogens, viruses, bacteria and nematodes is estimated to be US $2 billion. (Sharma et al., 2001). Yield losses due to drought are highly variable in nature depending on timing, intensity, and duration coupled with other location-specific environmental stress factors such as high irradiance and temperature. An annual estimated loss in groundnut production equivalent to over US$520 million are caused by drought. According to Johansen and Nigam (1994), almost half of it (US$208 million) can be recovered through genetic enhancement for drought resistance with a benefit:cost ratio of 5:2.

Drought affects the calcium uptake by pods and nitrogen fixation in groundnut where the photosynthesis is also reduced. When the crop reaches the harvesting stage there is the possibility of high contamination of the seeds with aflatoxins which makes the seeds toxic and unfit for consumption by both humans and livestock. It has been suggested by Freeman et al. (1999) that in the medium-term groundnut production and consumption is likely to shift increasingly to developing countries; production will grow in all regions but most rapidly in Asia, slowly in sub-Saharan Africa, and decline in Latin America; and there would be a shift in the utilization of groundnut products from oil to confectionery products suggesting an urgent need and shift in research for exploring the available genetic resources for their traits to tolerate drought.

Chickpea. Chickpea (*Cicer arietinum* L.) is the world's second most important pulse crop that is cultivated in more than 41 countries covering more than 11 million hectares of land and producing around 8 million tons of high protein food grain (FAO, 1999). The mean protein content of chickpea is about 24% that serves as an important source of dietary nutrition to poor of the SAT. It also has tremendous beneficial effects on increasing the productivity of succeeding crops in rotation besides enhancing the sustainability and productivity of production systems (Kumar Rao et al., 1998).

It is predominantly a rainfed crop grown after the end of the rains in south Asia and eastern Africa. This makes the crop to complete its life cycle on stored and receding soil moisture where it is exposed to increasing drought (Saxena, 2001). Despite having the yield potential of 5 t ha^{-1}, the average yield is only about 0.8 t ha^{-1} due to poor management practices and diseases. The yield losses across the SAT and the west Asia and north Africa (WANA) regions range from 40-60%. Global yield losses due to drought in chickpea are estimated to be around 3.7 million t and around 2.1 million t of these can be recovered through crop improvement efforts (Johansen et al., 1993). The yield losses in chickpea can be completely overcome with irrigation. Although some of the drought effects can be alleviated through agronomic and genetic options that do not involve external input of irrigation, these options can be only partial solution to this problem since the yield will always be lower than what can be achieved with irrigation (Saxena, 2001). It was shown that the yield gap between the most drought tolerant variety ICC 4958 and many other varieties with irrigation was large where the yield of ICC 4958 was only 30% of the potential, irrigated yield of most of the varieties. Conventional plant breeding techniques to upgrade the quality of the variety to tolerate drought have not been successful in chickpea due to the unavailability of appropriate methods to screen and breed varieties tolerant to drought, and also the appropriate selection tools to select the tolerant varieties.

Pigeonpea. Pigeonpea [*Cajanus cajan* L. (Millsp.)] is an important grain legume crop of the SAT that has ability to withstand drought conditions to produce grain of high protein concentration and biomass products such as fuel wood and fodder (Nene et al., 1990). It ranks sixth in area and production in comparison to other legumes of developing countries. It is widely grown in India mainly by resource-poor farmers accounting for 90% of the world production. Other regions where pigeonpea is grown are south east Asia, Asia, Africa, and the Americas. Its seed has approximately 21% of the protein content and is known as poor man's meat. The crop is usually grown under rainfed conditions where intermittent and terminal drought stress are frequent. It can be exposed to intermittent drought stress during dry periods of the normal rainy season and to terminal drought stress in the postrainy season. In recent years, the advent of shorter duration genotypes has widened the scope of pigeonpea cultivation in various, non-traditional cropping systems (Nam et al., 1993, 2000). Pigeonpea has an excellent deep and lateral rooted system that makes it salinity and alkalinity tolerant crop. Despite having such stress-tolerant traits in it, the yield and the productivity of pigeonpea, however, is very poor mainly due to the poor crop resource management (Lawn and Troedson, 1990).

While the traditional long duration pigeonpea can survive periods of drought stress during its growth cycle, the challenge is to improve the ability of the plant to produce under drought conditions. Studies in which irrigation has been given during the reproductive phase indicate that terminal drought usually reduces grain yield of landraces growing in their usual environment (Chauhan et al., 1992). A thorough characterization of the drought environments where pigeonpea is grown has not been adequately done so as to enable proper targeting of drought resistance traits (Johansen, 2001). There has been as yet no concerted breeding effort to enhance drought resistance of this crop due to the allocation of resources to other constraints like development of resistance to an important insect pest, *Helicoverpa armigera.*

Strategies to combat drought

Drought tolerance in plants is a complex trait and is mediated by many environmental adaptations that may involve many genes (Bohnert et al., 1995). A single strategy cannot confer drought tolerance in these plants. The problem of drought can best be tackled following a holistic approach integrating genetic resistance for drought, matching of genotypes to environments, and cultural methods that lead to better conservation and utilization of soil moisture. Further, efforts involving conventional and biotechnological approaches (see Figure 1) for genetic enhancement can certainly promise to induce and enhance the required drought tolerance in these important crop plants of SAT.

Conventional Approaches

a. Agronomic management options

Various aspects like providing supplementary irrigation, timing, method and intensity of irrigation would effect the yield of the crop (Wright and Nageshwara Rao 1994). Since in legumes the pod filling stage is very sensitive to drought, tackling the crop at such stage with proper care would increase the yield. The pod yield in groundnut can be increased by 13-19% if the crop is irrigated adequately during the pre-flowering phase (Nageshwara Rao et al., 1985b). The adaptive response of groundnut to drought can also be increased by exposing the crop to short duration drought during the vegetative phase which may enhance the root development and reduce transpirational losses by limiting the leaf area development allowing the plant to utilize the soil moisture from the deeper soil profile. This would reduce the impact of the second drought at the seedling stage by carefully selecting the cultivar, manipulating the sowing date, matching the water availability in the soil and intercropping with other crops can enhance the productivity and can ameliorate the severe effects of drought on groundnut productivity.

In chickpea too similar practices would have a positive impact on the crop productivity. However, being a post rainy crop and more sensitive to drought than groundnut few other simple input management options like improving the plant stand by timely sowing, using the high quality seeds, sowing the seed at soil depths so that it utilizes available soil moisture from the deeper regions of the soil profile, and seed dressing with fungicide which would increase the stability of plant stand per unit area (Saxena, 2001). Generally, sowing is done in surface layers where there is not adequate soil moisture after preparatory land cultivation. Advancing planting dates into autumn instead of traditional spring planting in WANA, and coinciding with late summer rains in south Asia and east Africa with disease tolerant varieties would allow plant to access to additional water concurrent with rainfall. Since the temperatures are low and evaporation is less during that rainy period, the evapotranspirational loss of water is reduced.

b.Plant breeding

In conjunction with the agronomic management, genetic management options in these crops can prove to be a better long-term strategy to improve the yield and performance of the crop in water limited and drought prone regions. At ICRISAT, genetic management approaches like developing short duration genotypes that can escape end-of-season drought, has resulted in 23% to 411% superior pod yield over the control varieties of groundnut (Nageshwara Rao and Nigam, 2001). Selecting the genotypes based on their fit to the historical weather and soil data of target location can also improve the overall efficiency of the crop. Alternatively genotypes which thrive well under limited moisture conditions show 12% to 144% pod yield superiority (see Nageshwara Rao and Nigam, 2001). Screening groundnut genotypes for better vegetative growth and pod yield under severe end-of-season and mid season drought has been an option. Genotypic variation for deep root system (Ketering 1984; Wright et al., 1991; Wright and Nageshwara Rao 1994), genotypes with lower mean SLA (surface leaf area) which have higher transcription efficiency has also been observed (Nageshwara Rao and Wright, 1994). Another way is to evaluate wild species for physiological traits associated with drought and aflatoxin resistance and identify suitable DNA markers for drought resistance gene[s] for use in inter-specific breeding to develop drought resistant lines (Nageshwara Rao and Nigam, 2001).

Conventional plant breeding with better agricultural management practices described above can promise to improve drought tolerance in the legume crops, however the major draw back of such conventional methods is that the available drought tolerant traits in the natural

system may not be readily amenable for breeding strategies due to species barrier and also the lacking of proper methods and techniques for screening the drought tolerant traits in the segregating populations thus obtained.

Biotechnological Approaches

Plant biotechnology offers new ideas and techniques applicable to agriculture. It uses the conceptual framework and technical approaches of plant tissue culture and molecular biology to develop commercial processes and products. These techniques enable the selection of successful genotypes, better isolation and cloning of favourable traits, and the creation of transgenic crops of importance to agriculture. This ability has moved agriculture from a resource-based to a science-based industry (Sharma and Ortiz, 2000). Together, these generic techniques are both an extension and an integral part of classical breeding, contributing successfully to shortening breeding and selection cycles (see Sharma et al., 2002). New bio-techniques, in addition to conventional plant breeding, are necessary to boost yields of the crops that feed the world (Borlaug, 1997), thereby contributing to food security by helping to promote sustainable agriculture centered on small-holder farmers in developing countries (Serageldin, 1999; Sharma and Ortiz, 2000). Although more difficult to control and engineer than the usually monogenic traits of resistance to biotic stresses, the genetically complex response to abiotic stress is globally and regionally far more important. Judicious application of biotechnological tools holds great potential in alleviating some of the major constraints to productivity of crops grown in the SAT. Schematic representation of the application of tools of biotechnology to address complex issues in agriculture such as drought stress is shown in Figure 1.

a. Molecular markers and marker-assisted selection

Many of the agronomic traits in plants are quantitative in nature. The process of domestication and selection have resulted in a drastic narrowing of the genetic variation of crop species (Tanksley and McCouch, 1997). The genetic/ physiology interaction provides a potential means of identifying candidate genes/ mechanisms (Forster et al., 2000). It is possible to identify and map the factors controlling characters as intransigent as yield (Thoday, 1961). Few loci in crops and their allelic forms account for a significant genetic variation in traits. Such loci can be mapped using molecular markers based on random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), DNA amplification polymorphism (DAF), sequence characterized amplified

regions (SCAR's), sequence-tagged sites (STS), expressed sequence tags (EST's), and amplicon length polymorphisms (ALPs) as long as polymorphism is observed in the segregating populations, the molecular marker is tightly linked to the trait of interest, and the phenotypic information on the population is available. Molecular markers have several advantages over the traditional phenotypic markers that were previously available to plant breeders. They offer great scope for improving the efficiency of conventional plant breeding by carrying out selection not directly on the trait of interest but on molecular markers linked to that trait. Moreover, these markers are not environmentally regulated and are, therefore, unaffected by the conditions in which the plants are grown and are detectable in all stages of plant growth (Mohan et al., 1997).

Although polymorphic variation in DNA has been detected in groundnut germplasm, the levels of genetic polymorphism have been very low (Bhagwat et al., 1997; He and Prakash, 1997; Hopkins et al., 1999; Subramanian et al., 2000). However, abundant polymorphism has been detected between related wild species in the section *Arachis* (Halward et al., 1991, 1992; Lanham et al., 1992; Paik-Ro et al., 1992). The information on biochemical and molecular basis for variation among the genotypes for drought resistance is very limited (Nageshwara Rao, et al., 1995). More recently, 43 drought-responsive mRNA transcripts (peanut transcripts responsive to drought; PTRD) that are up- or down-regulated following water stress have been identified in *Arachis hypogaea* (Jain et al., 2001). Amongst these 12 PTRD were completely suppressed due to prolonged drought while two were down-regulated, and two were up-regulated. Once the information in known, linkages can be developed between the drought resistance traits and the molecular markers to deploy the molecular tools to accelerate the plant breeding process for drought tolerant varieties.

The progress in genetic enhancement of chickpea and pigeonpea for drought tolerance is expected to progress faster with MAS for drought tolerance traits that would enhance the efficiency of breeding for this complex trait. According to Saxena (2001), the drought tolerant chickpea varieties already developed at ICRISAT and the segregating populations available for drought tolerant traits could be used to initiate such programs and build upon the information and material already available. However, genetic analysis particularly of quantitative traits associated with abiotic stress tolerance have associated errors. The detection of the loci involved is based on the probabilities and can be crude (Hay and Ellis, 1998), with different results in different environments. This approach may be limited to a few selective traits considering the practical difficulties involved in developing these genetic

markers for a given physiological trait and also the limitations associated with generating large numbers of early segregating materials to get a desired level of recombination if one has to handle several traits simultaneously in a breeding programme (Marshall, 1991; Subbarao et al., 1995). The application of molecular markers can promise more rigor when both the genetic and physiological analyses point to the same gene function, allowing greater confidence in identifying a candidate gene (Forster et al., 2000).

b. Transgenic technology and genes for drought tolerance

Genetic transformation provides a complementary means for the genetic betterment of the genome of field crops thus promising for alleviating some of the major constraints to crop productivity in developing countries (Sharma and Ortiz, 2000). The technology required for engineering transgenic plants is considerably more sophisticated than that required for production of hybrid plants by cross-fertilization (Goodman et al., 1987; Kung, 1993; Birch, 1997). Novel genes can be accessed from exotic sources-plants, animals, bacterial, even viral and introduced into a crop either physically through biolistics or by using binary vectors based in *Agrobacterium tumefaciens*. Further, it is possible to control the timing, tissue-specificity, and expression level of transferred genes for their optimal function. There are several traits whose correlative association with resistance has been tested in transgenic plants. The results of transgenic modifications of biosynthetic and metabolic pathways indicate that higher stress tolerance can be achieved by engineering, that tolerance is only marginally increased by the transfer of a single trait, and that multiple mechanisms to engineer water stress tolerance must be utilized (Bohnert et al., 1995).

Crops can be tailored to tolerate stress if only one knows the amount of damage that occurs to the plant at the physiological and cellular level. The physiological response to stress usually arises out of change in the cellular gene expression (Nanjo et al., 1999a). As a result changes in the integrity of the cellular membrane, imbalance in the homeostatic conditions and finally decline in the growth or death of the plant are usually noticed (Zhu, 2001). Such alteration in the gene expression is always involved in preparing the plant to thrive under stress. The distribution and occurrence of naturally stress-adapted plants in many different families like *Hydrilla verticillata, Najas indica*, *Najas graminia* belonging to submerged macrophytes (Rout and Shaw, 2001), the occurrence of stress- tolerant relatives in many glycophytic species like *Arabidopsis thaliana* (Zhu, 2001), and the occurrence of genetic variability in stress tolerance of crop plants per se makes the mechanisms that control the stress perception and the gene expression after the stress, universal in

plant kingdom (Bohnert et al., 1995).

Certain genes are expressed at elevated levels when a plant encounters stress (Bray, 1993). Hence, several gene transfer approaches have been employed to improve the stress tolerance of plants (Holmberg and Bulow, 1998; see also Fig. 1). These can be a product of single gene or a product of a regulatory gene that activates the whole cascade of other gene products in the plant in response to stress. Genetically engineered plants for single gene products include those encoding for enzymes required for the biosynthesis of osmoprotectants (Tarczynski et al., 1993; Kavikishore et al., 1995; Hayashi et al., 1997), or modifying membrane lipids (Kodama et al., 1994; Ishizaki-Nishizawa et al., 1996), LEA protein (Xu et al., 1996), and detoxification enzyme (McKersie et al., 1996). Similarly, many genes involved in stress response can be simultaneously regulated by using a single gene encoding stress-inducible transcription factor (Kasuga et al., 1999), thus offering possibility of enhancing tolerance towards multiple stresses including drought, salinity, and freezing. In general, very little attention has been given to genetic improvement to cope with intermittent drought (N.P. Saxena personal communication). It is believed that osmoregulation would be the best strategy for this kind of drought, especially if osmoregulatory genes could be triggered in response to drought. Some of the key strategies that have successfully been used in developing transgenic plants with some level of tolerance to abiotic stress are as follows:

Osmoprotectants: The maintenance of total water potential during water deficit can be achieved by osmotic adjustment. A reduction in cellular water potential below the external water potential, resulting from a decrease in osmotic potential, allows water to move into the cell. The osmotic potential inside the cell is lowered by the accumulation of osmoprotectants (compatible solutes) in the cytoplasm due to the induction of several genes (Bray et al., 1993). These are highly soluble compounds that carry no net charge at physiological pH and are non-toxic at high concentrations. Chemically, there are three types: betaines and allied compounds, polyols and sugars (e.g., betaines and trehalose), and amino acids such as proline (Pro) (Delauney and Verma, 1993; Stoop et al., 1996; McNeil et al., 1999). The first strategy involved in obtaining stress tolerant transgenic plants is to engineer such genes that encode enzymes for steps in the synthesis of these osmolytes. Various strategies are being pursued to genetically engineer increased osmoprotection in plants. Many osmoprotectants like glycine-betaine (Ishitani et al., 1995; McNeil et al., 2001), proline (Delauney and Verma, 1993; Nanjo et al., 1999a), choline oxidase (Sakamoto et al., 2000), glutamine synthetase (Hoshida et al., 2000), arginine decarboxylase (Roy and Wu, 2001), mannitol,

onnonitol, fructans, ectoine, and other gene products have been studied which play a role in osmoregulation thereby protecting the membrane and protein complexes (Yang et al., 1996). Although improved stress tolerance has not been achieved when native stress-induced genes are expressed, transgenic tobacco plants expressing a foreign gene leading to mannitol accumulation (Tarczynski et al., 1993) does improve stress tolerance. Free Pro is regarded as having multiple roles in stress tolerance in plants such as mediator of osmotic adjustment (Handa et al., 1986), a stabilizer of subcellular structures (Schobert and Tschesche, 1978), a scavenger of free radicals (Pardha Saradhi et al., 1995), a buffer in cellular redox potential and a major constituent of cell wall structural proteins that may provide mechanical support for cells (Nanjo et al., 1999b).

More recently, a positive correlation between Pro accumulation and stress tolerance in *Arabidopsis thaliana* has been shown by Nanjo et al. (1999a) by using antisense proline dehydogenase (*AtProDH*). Significant tolerance to drought stress has been observed in potato by using trehalose-6-phosphate synthase (TPS1) gene (Yeo et al., 2000). An improved protection of the photosynthetic apparatus was thought to be associated with the increased stress tolerance due to the introduced expression of *betA* gene that results in the production of glycine-betaine (Holmstrom et al., 2000).

Amongst the several groups of LEA proteins those belonging to the D-7 family are predicted to play a role in the sequestration of ions that are concentrated during cellular dehydration. These proteins have 11-mer amino acid motif with the concensus sequence TAQAAKEKAGE repeated as many as 13-times (Dure, 1993a). The D-19 family LEA proteins are predicted to have enhanced water-binding capacity, while the D-29 family is supposed to sequester ions during water loss. At least 30 different genes have been identified as members of lea group 2 (D-11 family) whose possible functions include a chaperone function or one that preserves protein structure (Dure, 1993b). However, further investigations combining physiological and genetic studies are needed to evaluate the role of water-deficit-induced genes and any adverse metabolic cost associated with osmoprotection.

Ion-Compartmentalization: Second strategy involves in protecting the crop against abiotic stress mainly salinity and water stress by regulating the ion uptake and compartmentalization of ions (Adams et al., 1992). These salts have detrimental effects on the biochemical processes of a plant resulting in the osmotic stress. Numerous metabolic changes occur in different salt sensitive plants which are subjected to ionic stress. The Na^+/H^+ exchange across the membrane is activated so that K^+ can be pumped across the cell membrane. Structurally plant cells are well suited for the

sequestration of ions because of the presence of the membrane-bound vacuoles. Many halophytes have a mechanism in them to compartmentalize Na^+ into vacuoles through a vacuolar Na^+/H^+ antiport. Such vacuolar Na^+/H^+ antiport activity was first reported in tonoplast vesicles from red beet storage tissue (Blumwald and Poole, 1985), and also in various halophytes and salt tolerant glycophytic species (Barkla and Pantoja, 1996). In *Arabidopsis* a vacuolar chloride channel, *AtCLCd* gene was cloned which involves in cation detoxification and more recently in *Arabidodpsis AtNHXI* gene which is homologous to *NhxI* gene in yeast has been cloned and over expressed in *Arabidopsis* which confers salt tolerance by compartmentalizing the Na^+ ions in the vacuoles. SOS1 (Salt Overly Sensitive 1) locus is one such locus in *Arabidopsis thaliana* which is similar to plasma membrane Na^+/H^+ antiport from bacteria and fungi and encodes a putative Na^+/H^+ antiporter that was cloned and over expressed using a CAMV35S promoter. *SOS1* gene expression in plants was up-regulated in response to salt tolerance (Shi et al., 2000).

Regulatory genes: Abiotic stress is a broad term which includes multiple stresses like heat, chilling, excessive light, drought, wounding, ozone exposure, UV-B irradiation, osmotic shock, where various cellular functions are disturbed. In order to restore the cellular function and make plant more tolerant to stress, transferring of a single gene encoding a single specific stress protein may not possibly show the required tolerance levels. To overcome such constraint enhancing tolerance towards multiple stress by a gene encoding a stress inducible cis-acting or trans-acting transcription factors that regulate a number of genes down stream or upstream of it thus activating a cascade of genes that act together in enhancing the tolerance towards the multiple stresses will prove to be a promising technology (Yamaguchi-Shinozaki et al., 1994; Kasuga et al., 1999). Both DREB1A cDNA and the *rd29A* promoter can be used to improve the dehydration, salt, and freezing tolerance of crops by gene transfer (Kasuga et al., 1999). Many genes respond to multiple stresses like dehydration and low temperature at the trascriptional level are also induced by ABA (Mundy and Chua, 1998) which protect the cell from dehydration. (Dure et al., 1989; Skriver and Mundy, 1990). Dehydration appears to trigger the production of ABA, which in turn induces expression of various genes (Ingram and Bartels, 1996; Shinozaki and Yamaguchi-Shinozaki, 1997). Transcription factors acting both *cis* as well as *trans* have been analyzed and a conserved sequence PyACGTGGC has been reported to function as ABA-responsive element (ABRE) (Marcotte et al., 1989). Genes which respond to these transcription factors or elements mostly have a basic domain and a leucine zipper structure (Guiltinan et al.,

1990; Oeda et al., 1991). Transgenic tobacco thus obtained by transferring the tetramer of a synthetic element conferred enhanced response to water stress (Lam and Chua, 1991).

There are other cis acting elements or factors of genes that have ABA independent expression (Yamaguchi-Shinozaki et al., 1992). A cis-acting element designated as dehydartion responsive element (DRE) having the sequence TACCGACAT is known to be involve in ABA-independent gene expression under drought, high salt, and low temperature conditions in many dehydration responsive genes like *rd29A* that is responsible for dehydration- and cold-induced expression. The cDNAs encoding the DRE-binding proteins, DREB1A and DREB2A, have been isolated and the proteins shown to specifically bind and activate transcription of genes (*rd29A*) containing the DRE sequence in *Arabidopsis* (Liu et al., 1998). For detail discussion on these regulatory genes and sequences see chapter by Shinozaki-Yamaguchi et al. in this report. Transforming such drought responsive elements and transcription factors such as DREB1A cDNA and *rd29A* promoter into crops like groundnut and chickpea using genetic transformation either by *Agrobacterium*-mediated transformation or by using a Biolistic approach, can be an effective strategy for the development of crops having improved tolerance to abiotic stress.

Current Status of legume transgenics at ICRISAT

Genetic engineering of plants makes it feasible to transfer genes from totally unrelated organisms thus breaking the species barrier which is otherwise not possible by conventional plant breeding. Gene transfer technologies have widened the scope for agriculture in modifying the crops to increase the yield, to impart resistance against various diseases, increase the nutritional content, and enhance resistance against drought. The application of this technology for the improvement of important crops of the SAT has been shown to hold great potential (Sharma and Ortiz, 2000). However, for the successful genetic modification by the production of transgenic plants, effective regeneration and transformation systems are imperative. Transformation of crops involves the stable introduction of DNA sequences into the nuclear genome of cells capable of giving rise to a whole transformed plant (Sharma and Ortiz, 2000). At ICRISAT, these barriers have recently been overcome and efficient transformation and regeneration of transgenic plants of the mandate legumes including groundnut, pigeonpea and chickpea is now routinely possible for various biotic and abiotic constraints (Sharma, 1999; Sharma and Anjaiah, 2000a,b).

Groundnut: The method for genetic transformation of groundnut (Sharma and Anjaiah, 2000b) reported from ICRISAT is a significant improvement over the previously reported results where at least 55% of the treated explants resulted in one or more independently transformed shoots. Multiple shoots originating from a single explant (Fig. 2A) showed different integration patterns suggesting that the transformation frequency in fact is higher. The transformation procedure utilizing cotyledon explants is highly susceptible to *Agrobacterium*-mediated gene transfer, and also displays very high regeneration rates across a wide range of *Arachis hypogaea* varieties of both Spanish and Virginia type peanuts. A key feature of this procedure is the regenerability of the cells wounded during excision of the cotyleodons at the time of culture initiation and Agro-infection, and these cells being mainly surface cells are readily accessible to the *Agrobacterium*. Shoot regeneration in this system is quite rapid and prolific, and a large proportion (>98%) of these shoots develop into phenotypically normal fertile plants where the transgene function is normal at transcriptional and translational levels. This system has been successfully utilized for the introduction of several genes such as those encoding for viral coat protein of Indian peanut clump virus (IPCV) and groundnut rosette assistor virus (GRAV), replicase of IPCV, *Bt Cry1A(b)*, and chitinase from rice. A new initiative with JIRCAS is being developed to utilize their constructs (rd29A:DREB1A) carrying DRE of *Arabidopsis* into *Arachis* for inducing drought resistance in groundnut. Putative transformants obtained in *Agrobacterium*-mediated transformation are being characterized for presence and expression of the introduced genes. The confirmed transgenic groundnut plants will be characterized for their phenotype and physiologically under normal and drought stress conditions to study their drought tolerance potential. The drought tolerance trait can eventually be transferred to selected genotypes with specific adaptations by conventional plant breeding by using the introduced genes as molecular markers. This protocol provides a transformation scheme that allows cost-effective, routine, and highly reproducible use of peanut transformation as part of both basic and applied studies in gene expression, transgenics, and functional genomics.

Pigeonpea: Efficient systems for the tissue culture regeneration of pigeonpea have been developed by using the axillary tissue (Fig. 2B; Sharma and Anjaiah, 2000a) and leaflet explants (Dayal and Sharma, unpublished results). A transformation protocol for pigeonpea to incorporate novel genes is in the final stages of development. A large number of putative transformants of pigeonpea have been successfully transferred to glasshouse and preliminary evidence suggests the presence of introduced genes like *Bt Cry 1A(b)*, soybean trypsin inhibitor (*SBTI*), and rice
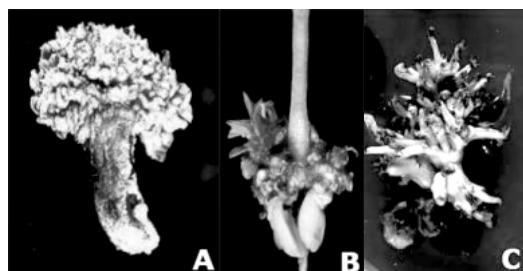
Fig. 2A-C. Tissue culture systems in legumes for use in developing abiotic stress tolerance. A. Regeneration of multiple shoots from cotyledon explants of *Arachis hypogaea*; B. Regeneration of multiple shoots from axillary meristems of *Cajanus cajan*; C. Regeneration of multiple shoots from axillary meristem explants of *Cicer arietinum*.

chitinase. Insect bioassays with tobacco transgenics having insecticidal genes have shown the potential of the available *Bt Cry1A(b)* and *SBTI* genes for insect control. Further work on developing resistance to fungal pathogens of pigeonpea is ongoing. These systems can be effectively used for developing drought resistant transgenics by employing the several strategies listed above.

Chickpea: A tissue culture system for chickpea has been optimized by using the embryo axis explants that produce multiple shoots (Fig. 2C). The axillary meristem tissues from embryo axis explants have been optimized to obtain a high frequency of shoots. Successful rooting and transplantation of these shoots is now possible with over 90% success rates. Putative transformants containing *Bt Cry 1A(b)* and *(SBTI)* genes have been obtained. Recently, this system is being employed to introduce genes for abiotic stress tolerance such as *codA* (Alia et al., 1999), *P5CSF:129A*, (Kavi Kishore et al., 1995), *mtld* (Tarczynski et al., 1993) and *annexin* (Gidrol et al., 1996). The putative transgenics are being analyzed at the molecular level.

Summary

Significant progress has been made in the field of agriculture to successfully enhance the drought tolerance in various plants using several strategies. The discovery of genes involved in osmoregulation, ion compartmentalization, and regulatory elements that are responsive to drought have opened up new avenues for developing crop plants with enhanced drought tolerance. Further research must be carried out in finding out the availability of stress responsive genes and transcripts in groundnut and chickpea and their expression under different environmental conditions. Wild species, due to their better adaptability offer unique opportunities for identifying and cloning new

genes and promoters that can be employed for developing stress tolerance by using an integrated approach as outlined in Figure 1. Gains in crop productivity through research advances in genetic enhancement will help to achieve sustainable food security, poverty alleviation, and environmental protection in the SAT. ICRISAT has a commitment to transfer the benefits of plant biotechnology to the developing world, as genes relevant to their crops and environment become available. Therefore, the research in transgenic crops offers a new means to achieve ICRISAT's mission, i.e., applying science to improve agriculture in areas of the world where sub-optimal rainfall and biotic stresses are the major constraints to crop productivity.

References

Adams, P., Vernon, D.M., Thomas, J.C., Bohnert, H.J., and Jensen, R.G., 1992. Distinct cellular and organismic responses to salt stress. Plant Cell Physiol., 33: 1215-1223.

Alia., Kondo, Y., Sakamoto, A., Nonaka, H., Hayashi, H., Pardhasaradhi, P., Tony H.H. Chen and Norio.M., 1999. Enhanced tolerance to light stress of transgenic *Arabidopsis* plants that express the *coaA* gene for a bacterial choline oxidase. Plant Mol. Biol., 40: 279-288.

Barkla, B.J and Pantoja, O., 1996. Physiology of ion transport across the tonoplast of higher plants. Annu. Rev. Plant Physiol. Plant Mol. Biol., 47: 159-184.

Bhagwat, A., Krishna, T.G. and Bhatia, C.R., 1997. RAPD analysis of induced mutants of groundnut (*A. hypogaea* L.). J. Genet., 76: 201-208.

Birch, R.G., 1997. Plant transformation: problems and strategies for practical application. Annu. Rev. Plant Physiol. Mol. Biol., 48: 297-326.

Blum, A., 1985. Crop varieties for stress environments. CRC Crit. Rev. Plant Sci., 2: 199-238.

Blumwald, E. and Poole, R.J., 1985. Na^+ / H^+ antiport in isolated tonoplast vesicles from storage tissue of *Beta vulgaris*. Plant Physiol., 78: 163-167.

Bohnert, H.J. and Jensen, R.G., 1996. Strategies for engineering water stress tolerance in plants. Trends Biotechnol., 14: 89-97.

Bohnert, H.J. and Shen, B., 1999. Transformation and

compatible solutes. Sci. Hort., 78: 237-260.

Bohnert, H.J., Nelson, D.E, Jensen, R.G., 1995. Adaptations to environmental stresses. Plant Cell, 7: 1099-1111.

Borlaug, N.E., 1997. Feeding a world of 10 million people: The miracle ahead. Plant Tiss. Cult. Biotech., 3:119-127.

Boyer, J.S., 1996. Advances in drought tolerance in plants. Adv. Agron., 56: 187-218.

Boyer, J.S., 1982. Plant productivity and environments. Science, 218: 443-447.

Bray, E.A., 1993. Molecular responses to water deficit. Plant Physiol., 103: 1035-1040.

Chauhan, Y.S., Saxena, N.P. and Johansen, C., 1992. Abiotic factors limiting chickpea and pigeonpea production. In: J.N. Sachan (ed.), Proc. Natl. Symp. on New frontiers in Pulses Research and Development, 10-12 November, 1989, Directorate of Pulses Research, Kanpur, India, pp. 111-123.

Delauney, A.J. and Verma, D.P.S., 1993. Proline biosynthesis and osmoregulation in plants. Plant J., 4: 215-223.

Dure, L. III, 1993a. A repeating 11-mer amino acid motif and plant desiccation. Plant J., 3: 363-369.

Dure, L. III, 1993b. Structural motifs in Lea proteins. In: T.J. Close and E.A. Bray (eds), Plant responses to cellular dehydration during environmental stress. Current Topics in Plant Physiology, Vol. 100. American Society of plant Physiologists, Rockville, MD, U.S.A., pp. 91-103.

Dure, L., III, Crouch, M., Harada, J., Ho., T.-h. D., Mundy, J., Quatrano, R., Thomas, T., and Sung, Z.R., 1989. Common amino acid sequence domains among the LEA proteins of higher plants. Plant Mol. Biol., 12: 475-486.

Edmeades, G.O., Bolanos, J., Banziger, M., Ribaut, J.M., White, J.W., Reynolds, M.P. and Lafitte, H.R., 1996. Improving crop yields under water deficits in the tropics. Proc. 2nd Intl. Crop Sci. Congress on crop productivity and Sustainability-Shaping the future, pp. 437-451.

FAO (Food and Agriculture Organization of the United Nations) 1999. FAO Production year book 1998. Rome, Italy: FAO.

Forster, B.P., Ellis, R.P., Thomas, W.T.B., Newton, A.C., Tuberosa, R., This, D., El-Enein, R.A., Bahri, M.H and Ben Salem, M., 2000. The development and application of molecular markers for abiotic stress tolerance in barley. J. Exp. Bot., 51: 19-27.

Freeman, H.A., Nigam, S.N., Kelly, T.G., N'tare, B.R., Subrahmanyam, P. and Boughton, D., 1999. The world groundnut economy: facts, trends, and outlook. International Crops research Institute for the Semi-Arid Tropics: Patancheru, Andhra Pradesh 502 324, India, p. 52.

Gidrol, X., Sabelli, P.A., Fern, Y.S. and Kush, A.K., 1996. Annexin-like protein from *Arabidopsis thaliana* rescue ΔoxyR mutant of *Escherichia coli* from H_2O_2 stress. Proc. Natl. Acad. Sci. USA, 93: 11268-11273.

Goodman, R.M., Hauptli, H. and Crossway, A. et al., 1987. Gene transfer in crop improvement. Science, 236: 48-54.

Guiltinan, M.J., Marcote, W.R., and Quatrano, R.S., 1990. A plant leucine zipper protein that recognizes an abscisic acid response element. Plant Physiol., 93: 267-271.

Halward, T.M., Stalker, H.T., LaRue, E. and Kochert, G., 1991.

Genetic variation detectable with molecular markers among unadapted germplasm resources of cultivated peanut and related wild peanut species. Genome, 34: 1013-1020.

Halward, T.M., Stalker, T., Larue, E. and Kochert, G., 1992. Use of single-primer DNA amplifications in genetic studies of peanut (*Arachis hypogaea* L.). Plant Mol. Biol., 18: 315-325.

Handa, S., Handa, A.K., Hasegawa, P.M. and Bressan, R.A., 1986. Proline accumulation and the adaptation of cultured plant cells to water stress. Plant Physiol., 80: 938-945.

Hanson, A.D. and Burnet, M., 1994. Evolution and metabolic engineering of osmoprotectant accumulation in higher plants. In: J.H. Cherry (ed.), Biochemical and cellular mechanism of stress tolerance in plants. Springer-Verlag, Berlin, pp. 105-124.

Hay, R.K.M and Ellis, R.P., 1998. The control of flowering in wheat and barley: what recent advances in molecular genetics reveal. Ann. Bot., 82: 541-554.

Hayashi, H., Mustardy, L., Deshnium, P., Ida, M and Murata, N., 1997. Transformation of *Arabidopsis thaliana* with the *codA* gene for choline oxidase: accumulation of glycine-betaine and enhanced tolerance to salt and cold stress. Plant J., 12: 133-142.

He, G. and Prakash, C.S., 1997. Identification of polymorphic DNA markers in cultivated peanut (*Arachis hypogaea* L.). Euphytica, 97: 143-149.

Holmber, N. and Bulow, L., 1998. Improving stress tolerance in plants by gene transfer. Trends Plant Sci., 3: 61-66.

Holmstrom, K.O., Somersalo, S., Mandal, A., Palva, T.E. and Welin, B., 2000. Improved tolerance to salinity and low temperature in transgenic tobacco producing glycine betaine. J. Exp. Bot., 51: 177-185.

Hopkins, M.S., Casa, A.M., Wang, T., Michell, S.E., Dean, R.E., Kochert, G.D. and Kresovich, S., 1999. Discovery and characterization of polymorphic simple sequence repeats (SSRs) in peanut. Crop Sci., 39: 1243-1247.

Hoshida, H., Tanaka, Y., Hibino, T., Hayashi, Y., Tanaka, A., Tanaka, T., Takabe, T., 2000. Enhanced tolerance to salt stress in transgenic rice that overexpress chloroplast glutamine synthetase. Plant Mol Biol., 43: 103-111.

Ingram, J. and Bartels, D., 1996. The molecular basis of dehydration tolerance in plants. Annu. Rev. Plant Physiol. Mol. Biol., 47: 377-403.

Ishitani, M., Nakamura, T., Hans, S.Y., Takabe, T., 1995. Expression of betaine aldehyde dehydrogenase gene in barley in response to osmotic stress and absicsic acid. Plant. Mol. Biol., 27: 307- 315.

Ishizaki-Nishizawa, O., Fujii, T., Azume, M., Sekiguchu, K., Murata, N., Ohtani, T. and Toguri, T., 1996. Low temperature resistance of higher plants is significantly enhanced by a nonspecific cyanobacterial desaturase. Nat. Biotechnol., 14: 1003-1006.

Jain, A.K., Basha, S.M. and Holbrook, C.C., 2001. Identification of drought-responsive transcripts in peanut *(Arachis hypogaea* L.). Electronic J. Biotech. Vol. 4 [http://www.ejb.org/content/vol4/issue2/full/2/index.html]

Johansen, C., 2001. An overview of prospects for genetic enhancement of drought resistance in pigeonpea. In: N.P. Saxena (ed.), Management of Agricultural Drought - Agronomic and Genetic Options. Oxford & IBH Publishing Co. Pvt. Ltd.: New Delhi. (in press).

Johansen, C. and Nigam, S.N., 1994. Importance of drought stress and its alleviation in legumes. Crop Sci., 24: 17-19.

Johansen, C., Baldev, B., Brouwer, J.B., Erskine, W., Jermyn, W.A., Lang L.J., Maik, B.A., Ahad Miah, A. and Silim, S.N., 1993. Biotic and abiotic stresses constraining productivity of cool season food legumes in Asia, Africa, and Oceania. In: F.J. Mehbauer and W.J. Kaiser (eds), Proc. Intl. Food Legumes Research Coference II, 12-16 April, 1992, Kluwer: Dordretcht.

Kasuga, M., Liu, Q., Miura, S., Yamaguchi-Shinozaki, K. and Shinozaki, K., 1999. Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. Nat. Biotechnol., 17: 287-291.

Kavi Kishore, P.B., Hong, Z., Miao, G.-U., Hu, C.-A.H. and Verma, D.P.S., 1995. Overexpression of Δ'-pyrroline-5-carboxylate synthase increases proline production and confers osmotolerance in transgenic plants. Plant Physiol., 108: 1387-1394.

Ketering, D.L., 1984. Root Diversity among peanut genotypes. Crop Sci., 24: 229-232.

Kodama, H., Hamada, T., Horiguchi, G., Nishimura, M. and Iba, K., 1994. Genetic enhancement of cold tolerance by expression of a gene for chloroplast Ω-3 fatty acid desaturase in transgenic tobacco. Plant Physiol., 105: 601-605.

Kramer, P. J., 1980. The role of physiology in crop improvement. In: R.C. Staples and R.J. Kuhr (eds), Linking Research to Crop Production, Plenum Press, New York, pp. 51-62.

Kung, S.D., 1993. Introduction: from hybrid plants to transgenic plants. In: S.D. Kung and R. Wu (eds), Transgenic plants, engineering and utilization, vol. 1. Academic Press: San Diego, U.S.A., pp. 1-12.

Kumar Rao, J.V.D.K., Johansen, C. and Rego, T.J., 1998. Concluding remarks. In: J.D.V.K. Kumar Rao, C. Johansen, T.J. and Rego (eds), Residual effects of legumes in rice and wheat cropping systems of the Indo-Gangetic plain. Oxford & IBH Publ. Co.: New Delhi, pp. 237-240.

Lanham, P.G., Fennell, S., Moss, J.P. and Powell, W., 1992. Detection of polymorphic loci in *Arachis* germplasm using random amplified polymorphic DNAs. Genome, 35: 885-889.

Lam, E., and Chua, N-H., 1991. Tetramer of a 21-base pair synthetic element confers seed expression and trasncriptional enhancement in response to water stress and abscisic acid. J. Biol. Chem., 266: 17131-17135.

Lawn, R.J and Troedson, R.J., 1990. Pigeonpea: Physiology and yield formation. CAB International: Wallingford, U.K., pp. 179-208.

Liu, Q., Kasuga, M., Sakuma, Y., Abe, H., Miura, S., Yamaguchi-Shinozaki, K. and Shinozaki, K., 1998. Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively, in Arabidopsis. Plant Cell, 10: 1391-1406.

Ludlow, M.M., 1993. Physiological mechanisms of drought resistance. In: T.J. Mabry, H.T. Nguyen, R.A. Dixon and M.S. Bonness (eds), Biotechnology for arid land plants. IC^2 Institute, The University of Texas at Austin, USA, pp. 11-34.

Marcotte, W.R., Jr., Russel, S.H., and Quatrano, R.S., 1989. Abscisic acid-response sequences from the *Em* gene of wheat. Plant Cell, 1: 969-976.

Marshall, D.R., 1991. Alternative approaches and perspectives in breeding for higher yields. Field Crops Res., 26: 171-190.

Mckersie, B.D., Bowley, S.R., Harjanto, E. and Leprince, O., 1996. Water deficit tolerance and field performance of transgenic alfalfa overexpressing superoxide dismutase. Plant Physiol., 111: 1177-1181.

McNeil, S.D., Nuccio, M.L. and Hanson, A.D., 1999. Betaines and related osmoprotectants. Targets for metabolic engineering of stress resistance. Plant Physiol., 120: 945-949.

McNeil, S.D., Nuccio, M.L., Ziemak, M.J. and Hanson, A.D., 2001. Enhanced synthesis of choline and glycine betaine in transgenic tobacco plants that overexpress phosphoethanolamine N-methyltransferase. Proc. Natl. Acad. Sci. USA, 98: 10001-10005.

Mohan, M., Nair, S., Bhagwat, A., Krishna, T.G., Yano, M., Bhatia, C.R. and Sasaki, T., 1997. Genome mapping, molecular markers and marker-assisted selection in crop plants. Mol. Breed., 3: 87-103.

Mundy, J., and Chua, N.H., 1998. Abscisic acid and water stress induce the expression of novel rice gene. EMBO J., 7: 2279-2286.

Nageshwara Rao, R.C. and Nigam, S.N., 2001. Genetic options for drought management in groundnut. In: N.P. Saxena (ed.), Management of Agricultural Drought - Agronomic and Genetic Options. Oxford & IBH Publishing Co. Pvt. Ltd.: New Delhi. (in press).

Nageshwara Rao, R.C., Singh, S., Sivakumar, M.V.K., Srivastava, K.L., and Williams, J.H., 1985a. Effect of water deficit at different growth phases of peanut. I. Yield responses. Agron. J., 77, 782-786.

Nageshwara Rao, R.C., Singh, S., Sivakumar, M.V.K., Srivastava, K.L., and Williams, J.H., 1985b. Effect of water deficit at different growth phases of peanut. II. Response to drought during pre-flowering phase. Agron. J., 80, 431-438.

Nageshwara Rao, R.C., and Wright, G.C., 1994. Stability of the relationship between specific leaf area and carbon isotope discrimination across environments in peanut. Crop Sci., 34: 98-103.

Nageshwara Rao, R.C., Udayakumar, M., Farquhar, G.D., Talwar, H.S., and Prasad, T.G., 1995. Variation in carbon isotope discrimination and its relationship to specific leaf area and ribulose-1, 5 bisphosphate carboxylase content in groundnut genotypes. Aust. J. Plant Physiol., 22: 545- 551.

Nam, N.H., Chauhan, Y.S. and Johansen, C., 1993. Comparison of extra-short duration pigeonpea with short-season legumes under rainfed conditions on Alfisols. Exp. Agri., 29: 307-316.

Nam, N.H., Chauhan, Y.S. and Johansen, C., 2000. Effects of timing of drought stress on extra-short duration pigeonpea. J. Agric. Sci., Cambridge, (in press).

Nanjo, T., Kobayashi, M., Yoshiba, Y., Kakubari, Y., Yamaguchi-Shinozaki, K. and Shinozaki, K., 1999a. Antisense supression of proline degradation improves tolerance to freezing and salinity in *Arabidopsis thaliana*. FEBS Lett., 461: 205-210.

Nanjo, T., Kobayashi, M., Yoshiba, Y., Sanada, Y., Wada, K., Tsukaya, H., Kakubari, Y., Yamaguchi-Shinozaki, K. and Shinozaki, K., 1999b. Biological functions of proline in morphogenesis and osmotolerance revealed in antisense transgenic *Arabidopsis thaliana*. Plant J., 18: 185-193.

Nene, Y.L., and Sheila, V.K., 1990. Pigeonpea: Geography and Importance. In: Y.L Nene, S.D. Hall and V.K. Sheila (eds), The Pigeonpea. CAB Intl: Wallingford, U.K., pp. 1-14.

Nguyen, H.T., Babu, R.C. and Blum, A., 1997. Breeding for drought resistance in rice: physiology and molecular genetics considerations. Crop. Sci., 37: 1426-1434.

Oeda, K., Salinas, J., and Chua, N.-H., 1991. A Tobacco bZip transcription activator (TAF-1) binds to a G-box- like motif conserved in plant genes. EMBO J., 10: 1793-1802.

Paik-Ro, O.G., Smith, R.L. and Knauft, D.A., 1992. Restriction fragment length polymorphism evaluation of six peanut species within the *Arachis* section. Theor. Appl. Genet., 84: 201-208.

Pardha Saradhi, P., Alia, Arora, S. and Prasad, K.V.S.K., 1995. Proline accumulates in plants exposed to UV radiation and protects them against UV induced peroxidation. Biochem. Biophys. Res. Commun., 201: 1-5.

Peng, Z., Lu, Q., Verma, D.P., 1996. Reciprocal regulation of delta1-pyrroline-5-carboxylate synthetase and proline dehydrogenase genes controls proline levels during and after osmotic stress in plants. Mol. Gen. Genet., 253: 334-341.

Pilon-Smits, E.A.H., Ebskamp, M.J.M., Paul, M.J., Jeuken, J.W., Weisbeek, and Smeekens, S.C.M., 1995. Improved performance of transgenic fructan-accumulating tobacco under drought stress. Plant Physiol., 107: 125- 130.

Ribaut, J.M., Hosington, D.A., Deitech, J.A., Jiang, C. and Gonzalex-de-Leon, D., 1996. Identification of quantitative trait loci under drought conditions in tropical maize. 1. Flowering parameters and the anthesis-silking interval. Theor. Appl. Genet., 92: 905-914.

Ribaut, J.M., Jiang, C., Gonzalex-de-Leon, D., Edmeades, G.O. and Hosington, D.A., 1997. Identification of quantitative trait loci under drought conditions in tropical maize. 2. Yield components and marker-assisted selection strategies. Theor. Appl. Genet., 94: 887-896.

Rout, N.P and Shaw, B.P., 2001. Salt tolerance in aquatic macrophytes: Possible involvement of the anti oxidative enzymes. Plant Sci., 160: 415-423.

Roy, M. and Wu, R., 2001. Arginine decarboxylase transgene expression and analysis of environmental stress tolerance in transgenic rice. Plant Sci., 160: 869-875.

Sakamoto, A., Valverde, R., Alia., Chen, T.H., Murata, N., 2000. Tranformation of Arabidopsis with the *codA* gene for choline oxidase enhances freezing tolerance of plants. Plant J., 22: 449-453.

Saxena, N.P., 2001. Management of drought in chickpea- a holistic approach. In: N.P. Saxena (ed.), Management of Agricultural Drought - Agronomic and Genetic Options. Oxford & IBH Publishing Co. Pvt. Ltd.: New Delhi. (in press).

Schobert, B. and Tschesche, H., 1978. Biochim. Biophys. Acta, 541: 270-277.

Serageldin, I., 1999. Biotechnology and Food Security in the 21st century. Science, 285: 387-389.

Sharma, H.C., Sharma, K.K., Seetharama, N. and Ortiz, R., 2001. Genetic transformation of crop plants: Risks and opportunities for the rural poor. Curr. Sci., 80, 1495-1508.

Sharma, K.K., 1999. Genetic transformation of groundnut at ICRISAT. http://www.icrisat.org/text/research/grep/homepage/archives_cd/archives/gtl.htm.

Sharma, K.K. and Anjaiah, V., 2000a. Genetic Transformation of pigeonpea at ICRISAT. http://198.93.234.24/homepage/archives_cd/archives/gtppatic.htm.

Sharma, K.K and Anjaiah, V., 2000b. An efficient method for the production of transgenic plants of peanut *(Arachis hypogaea* L.) through *Agrobacterium tumefaciens*-mediated genetic transformation. Plant Sci., 159: 7-19.

Sharma, K.K. and Ortiz, R., 2000. Program for the application of genetic transformation for crop improvement in the semi-arid tropics: In Vitro Cell. Dev. Biol.-Plant, 36: 83-92.

Sharma, K.K., Sharma, H.C., Seetharama, N. and Ortiz, R., 2002. Development and deployment of transgenic plants: Biosafety considerations. In Vitro Cell. Dev. Biol.-Plant 86: 1-11.

Shi, H., Ishitani, M., Kim, C. and Zhu, J.K., 2000. The *Arabidopsis thaliana* salt tolerance gene *SOS1* encodes a putative Na$^+$ / H$^+$ antiporter. Proc. Nat. Acad. Sci. USA.97: 6896-6901.

Shinozaki, K. and Yamaguchi-Shinozaki, K., 1997. Gene expression and signal transduction in water-stress response. Plant Physiol., 115: 327-334.

Sivamani, E., Bahieldin, A., Wraith, J.M., Al-Niemi, T., Dyer, W.E., Ho, T.H.D., Qu, R., 2000. Improved biomass productivity and water use efficiency under water deficit conditions in transgenic wheat constitutively expressing the barley *HVA1* gene. Plant Sci., 155: 1-9.

Skriver, K., and Mundy, J., 1990. Gene expression in response to abscisic acid and osmotic stress. Plant Cell, 5: 503-512.

Stoop, J.M.H., Williamson, J.D. and Pharr, D.M., 1996. Mannitol metabolism in plants: a method for coping with stress. Trends Plant Sci., 1: 139-144.

Subbarao, G.V., Johansen, C., Slinkard, A.E., Nageshwara Rao, R.C., Saxena, N.P. and Chauhan, Y.S., 1995. Strategies for improving drought resistance in grain legumes. Crit. Rev. Plant Sci., 14: 469-523.

Subramanian, V., Gurtu, S., Nageshwara Rao, R.C and Nigam, S.N., 2000. Identification of DNA polymorphism in cultivated groundnut using random amplified polymorphic DNA (RAPD) assay. Genome, 43: 656-660.

Tanskley, S.D., McCouch, 1997. Seed banks and molecular maps: unlocking genetic potential from the wild. Science, 227: 1063-1066.

Tarczynski, M.C., Jensen, R.G., Bohnert, H.J., 1993. Stress protection of transgenic tobacco by production of osmolyte mannitol. Science, 259: 508-510.

Thoday, J.H., 1961. Location of polygenes. Nature, 191: 368-370.

Udaya Kumar, M., Sheshashayee, M.S., Nataraj, K.N., Bindu Madhava, H., Devendra, R., Aftab Hussain, I.S. and Prasad, T.G., 1998. Why has breeding for WUE not been successful? An analysis and alternate approaches to exploit this trait for crop improvement. Curr. Sci., 74: 996-1000.

Virmani, S.M. and Singh, P., 1986. Agroclimatological characteristics of the groundnut-growing regions in the semi-arid tropics. Proceedings of international symposium ICRISAT, INDIA, 35-46.

Wright, G.C and Nageshwara Rao, R.C., 1994. Groundnut water relations. In: J. Smart (ed.), The Groundnut Crop: A scientific basis for improvement, Chapman & Hall, London, U.K., pp.281- 335.

Wright, G.C., Hubick, K.T., and Farquhr, G.D., 1991. Physiological analysis of peanut cultivar response to timing and duration of drought stress. Aust. J. Agric. Res., 42: 453-470.

Xu, D., Duan, X., Wang, B., Hong, B., Ho, T.-H.D. and Wu, R., 1996. Expression of a late embryogenesis abundant protein gene, *HVA1*, from barley confers tolerance to water deficit and salt stress in transgenic rice. Plant Physiol., 110: 249-257.

Yamaguchi-Shinozaki, K., Koizumi, M., Urao S., and Shinozaki, K., 1992. Molecular cloning and characterization of 9 cDNAs for genes that are responsive to dessication in *Arabidopsis thaliana*: Sequence analysis of one cDNA clone that encodes a putative trans-membrane channel protein. Plant Cell Physiol., 33: 217-224.

Yamaguchi-Shinozaki, K., Urao, T., Iwasaki, T., Kiyosue, T. and Shinozaki, K., 1994. Function and regulation of genes that are induced by dehydration stress in *Arabidopsis thaliana*. JIRCAS J., 1: 69-79.

Yang, G., Rhodes, D. and Joly, R.J., 1996. Effects of high temperature on membrane stability and chlorophyll fluorescence in glycinebetaine-deficient and glycinebetaine-containing maize lines. Aust. J. Plant Physiol., 23: 437-443.

Yeo, E.T., Kwon, H.B., Han, S.E., Lee, J.T and Byu, M.O., 2000. Genetic engineering of drought resistant potato plants by introduction of the trehalose-6-phosphate synthase (TPS1) gene from *Saccharomyces cerevisiae*. Mol Cells, 30: 263-268.

Zhang, J.X., Nguyen, H.T. and Blum, A., 1999. Genetic analysis of osmotic adjustment in crop plants. J. Exp. Bot., 50: 291-302.

Zhu, J.K., 2001. Plant salt tolerance. Trends Plant Sci., 6: 66-71.