Journal of Food Legumes 24(3): 165-174, 2011

Breeding for abiotic stresses in pigeonpea

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(Received: June 30, 2011; Accepted: August 20, 2011)

ABSTRACT

Pigeonpea, often considered as a drought tolerant crop, has the distinct advantage of having a large range of variation for maturity, leading to its adaptation to a wide range of environments and cropping systems. It encounters a number of abiotic stresses during its life cycle. The most important are extremes of moisture and temperature, photoperiod and mineral related stresses. While waterlogging affects plant growth by reducing oxygen diffusion rate between soil and atmosphere and by changing physical and chemical properties of soil, drought and high temperature mostly influence long duration pigeonpea, resulting in its forced maturity. Similarly, low temperature leads to conversion of intracellular water into ice and consequently shrinking of cells and wilting and death of plants. Soil salinity affects pigeonpea plants through osmotic stress and interference with uptake of mineral nutrients. Aluminium toxicity also reduces nutrient uptake efficiency of this crop. Though these stresses have a drastic impact on reducing productivity of pigeonpea, only limited efforts have been made towards screening and development of pigeonpea genotypes having tolerance to these abiotic stresses. Further, even these limited accomplishments are not well-documented. The present review provides comprehensive information vis-a-vis the work done on abiotic stress tolerance in pigeonpea.

Key words: Abiotic stresses, Aluminium toxicity, *Cajanus cajan*, Drought tolerance, Low temperature, Mineral stress, Photoperiod, Pigeonpea, Waterlogging

Pigeonpea [*Cajanus cajan* (L.) Millspaugh] is one of the major food legume crops of the tropics and sub-tropics. In India, after chickpea, pigeonpea is the second most important pulse crop. It is mainly eaten in the form of split pulse as 'dal'. Despite its main use as de-hulled split peas, the use of immature seeds is very common as fresh vegetable in some parts of India such as Gujarat, Maharashtra and Karnataka. Besides this, in the tribal areas of various states, the use of pigeonpea as green vegetable is very common (Saxena *et al.* 2010).

Pigeonpea is grown in a number of countries of Asia, Eastern and Southern Africa, Latin America and Caribbean countries. Globally, it is cultivated on 4.92 million ha (mha) with an annual production of 3.65 million tons (mt) and productivity of 898 kg/ha (http://www.icrisat.org). India has the largest acreage under pigeonpea (3.90 mha) with a total production and productivity of 2.89 mt and 741 kg/ha, respectively (DAC 2011).

Pigeonpea is considered as a drought tolerant crop with a large variation for maturity period. As a result, it is widely adapted to a range of environments and cropping systems. Broadly, four maturity groups are recognized in pigeonpea: extra early (90 – 120 days), early (120 – 150 days), medium (150 – 200 days), and late (200 – 300 days). These variations for maturity have direct relevance on the survival and fitness of the crop in different agro-ecological niches (Choudhary 2011).

Pigeonpea encounters various abiotic stresses during its life cycle such as moisture (waterlogging/drought), temperature, photoperiod and mineral (salinity/acidity) stress. Among these stresses, moisture stress is common because pigeonpea is generally grown as a rain-fed crop. In the north-western and north-eastern parts of India, the stress imposed by extremes of temperature (too low/ too high) during the reproductive stage often leads to crop failure. Some areas in these regions have acidic soils with problems of aluminium toxicity. In some other areas such as parts of Haryana and Punjab, the crop suffers from both waterlogging and soil salinity stresses. Empirical evidences reveal that one abiotic stress is often linked with several other stresses, making it difficult to know the exact cause of the crop failure. The literature pertaining to screening, identification of tolerant genotypes and their utilization for improving tolerance to such abiotic stress in pigeonpea is scanty and also not well-documented as compared to cereals. Therefore, the present paper aims to review the available information on abiotic stresses and discusses the ways to improve the tolerance to these stresses in pigeonpea.

A. Waterlogging

Non-wetland crop species require well-drained soils for optimal growth and production of yield. Prolonged water saturation has a significant impact on both biotic and abiotic attributes of the soils. Drastic reduction in oxygen partial pressure is the primary plant stress under excessive moisture condition. Inability of non-wetland crop species (including pigeonpea) to withstand low oxygen conditions in rhizosphere, caused by waterlogging or any other factor, results in substantial yield losses. Roots of most plants are highly sensitive to anaerobic conditions, which support a unique

microbial community compared to aerobic conditions, and this severely affects the nutrient relation of the soil. Shortly after the onset of waterlogging conditions (2-3 days), obligate aerobic bacteria become inactive, and facultative/obligate anaerobic bacteria become active and dominate the micro-flora/fauna of inundated soils. Over time, the activity of anaerobic bacteria causes sharp decline in redox potential, which causes severe nutrient imbalances. Another important side effect of excessive moisture is leaching of mineral nutrients and/or essential intermediate metabolites from the roots into the volume of water in which they are immersed. Excessive soil moisture causes major changes in physical and chemical properties in rhizosphere. Oxygen diffusion rates (ODR) in flooded soil is about 100 times lower than air (Kennedy *et al*. 1992), and respiration of plant roots, soil micro-flora and fauna leads to rapid exhaustion of soil oxygen, and thereby causing anaerobiosis. Oxygen deprivation, either completely (anoxia) or partially (hypoxia) is detrimental to most species of higher plants as it disturbs the respiratory cycle of plant by changing it from aerobic (kreb cycle = 38 ATP) to anaerobic (glycolysis=2 ATP) cycle (Armstrong *et al.* 1994). However, proximate causes of plant injury can be oxygen deficit or mineral nutrient imbalances, a decrease in cytokinins or other hormones released from the roots, a decrease in available soil nitrogen and/or nitrogen uptake, an increase in toxic compounds in soil such as methane, ethylene, ferrous ions or manganese, an increase in toxic compounds (in the plant) such as ethanol or ethylene, and an increase in disease causing organisms.

The areas where rainfall is dependent on monsoon are more prone to waterlogging. Waterlogging occurs when rainfall or irrigation water is collected on the soil surface for prolonged periods without infiltrating into the soil. Soil characteristics that contribute to waterlogging include its physical properties that allow formation of a crust on the soil surface or a subsoil pan particularly those of high water holding capacity soils, such as vertisols and Indo-Gangetic alluvial soils (Reddy and Virmani 1980). Waterlogging can also occur when the amount of water added through rainfall or irrigation is more than what can percolate into the soil within one or two days. This condition sometimes results in rise of water table, causing development of salinity/alkalinity as observed in Haryana and the Punjab.

In India, waterlogging is one of the most serious constraints for crop production and productivity, where about 8.5 mha of arable land is prone to this problem. Out of the total (3.9 mha) area under pigeonpea, about 1.1 mha is affected by excess soil moisture, causing an annual loss of 25-30% (Sultana 2010).

Pigeonpea is primarily grown between 14º and 30º latitudes where the mean annual rainfall ranges between 600 and 1500 mm. Waterlogging is a major production constraint in these regions particularly in deep vertisols. Some of the important pigeonpea growing areas where severe waterlogging is encountered are Yavtmal and the adjoining areas in Maharashtra, Gorakhpur, Faizabad, Varanasi, Jaunpur, Kanpur, Ghazipur and Fatehpur in Uttar Pradesh, Jabalpur in Madhya Pradesh, Surat and Navsari in Gujarat and northern and eastern regions of Bihar.

Fig 1. Pigeonpea genotypes showing chlorosis /nitrogen deficiency after receiving heavy rainfall continuously for 10 days at early seedling stage at ICRISAT, Patancheru during 2009

The risk of crop failure or yield reduction due to waterlogging is quite high in extra-early and early duration varieties because they get less time to recover from this stress as compared to medium and long duration varieties (Matsunaga *et al*. 1991). In pigeonpea, a few studies have shown the relationship between waterlogging and severity of diseases caused by *Phytophthora* species (RHS 2010). In general, waterlogging conditions increased the incidence of root rot caused by *Fusarium cactum*. High soil moisture directly favours *Phytophthora* species by providing a continuous water film necessary for zoospore production and their motility. In addition, high soil water may indirectly favour infection and colonization of the host tissues by the fungus by decreasing host vigour and increasing the release of host exudates that may stimulate germination of dormant propagules (Duncan *et al.* 2007).

Waterlogging can affect pigeonpea during germination, early and late seedling stages as these stages cover peak monsoon period (Fig. 1). These three critical stages can be used for screening of tolerant genotypes. Empirical evidences suggest that waterlogging causes rapid senescence and drooping of the shoot tips of plants. It reduces plant height and delays flowering in surviving plants, resulting in reduction in the number of pods, seeds/pod and seed yield. It has been observed that seed coat thickness, aerenchymatous cells, lenticels and adventitious roots also affect tolerance to waterlogging in pigeonpea. However, these traits need to be confirmed and re-validated before these can be used as selection criteria in pigeonpea.

Preliminary screening of pigeonpea cultivars and genotypes for waterlogging tolerance has been done at ICRISAT (Patancheru, Hyderabad), IIPR (Kanpur) and Institute of Agricultural Sciences (BHU, Varanasi). Some tolerant cultivars and advance breeding lines have been identified (Table 1).

Perera *et al.* (2001) studied the genetics of different morphological traits. The results showed that the additive and dominant gene effects control the expression of most of the important traits. It was also observed that crosses between the tolerant and sensitive lines showed more genetic variation than those among tolerant lines, suggesting potential for genetic improvement for these traits. Sarode *et al.* (2007) worked out genetics and identified the gene for waterlogging tolerance in pigeonpea. They studied segregation pattern in two crosses derived from tolerant parent 'ICPL 84023' and sensitive parents 'DA 11' and 'MA 98 PTH 1'. Their study revealed that waterlogging tolerance is a dominant trait and is governed by a single gene. Therefore, it can easily be transferred by backcrossing to leading varieties, which are sensitive to waterlogging. However, it could be possible that parents might be differing at a single locus. Therefore, they suggested making more efforts involving a large number of tolerant and sensitive parents to understand the genetic control of waterlogging tolerance. This may be helpful in breeding waterlogging tolerant varieties, so that the crop efficiency can be increased and the true potential of pigeonpea as pulse crop can be fully exploited.

There is need to standardize screening techniques against excessive soil moisture in pigeonpea. This calls for large-scale utilization of pigeonpea germplasm for re-validation of screening techniques (strategic research). Identification of gene(s) conferring waterlogging tolerance (basic research) and its incorporation into the cultivars (applied research) to have viable outcome (anticipatory research) merit special attention. Besides, adaptation mechanisms to waterlogging tolerance also need to be explored.

B. Drought Stress

Pigeonpea is grown in *kharif* season as a rainfed crop. It is considered as a drought tolerant legume on account of its deep root system. Among the four maturity groups, extra-early and early types complete their life cycle just after recession of the monsoon season. However, their reproductive phase more often encounters terminal drought. The situation becomes even worse for medium and long-duration pigeonpea as their flowering and pod-filling stages coincide acute soil moisture deficit in absence of any supplementary irrigation.

Likoswe and Lawn (2008) studied species differences in drought response of three grain legumes, namely soybean, cowpea and pigeonpea to assess how water deficit affects water use, growth and survival of plants in pure stand and in competition. In pure stand, pigeonpea appeared to be slow, tolerant and slow for the rate of plant available water (PAW) depletion, no. of nodes and node growth and senescence of the lower leaves, respectively. Cowpea and pigeonpea extracted almost all PAW and died after an average of 18 days and 14 days, respectively following maximum PAW depletion. In contrast, soybean died before 90% of PAW was depleted and so in pure stand used less water. There were otherwise only minor differences between the species combinations in the timing and maximum level of PAW depletion. The ability of cowpea and pigeonpea to maintain leaf water status above lethal levels for longer was achieved through different means. Pigeonpea appeared to rely primarily on *dehydration tolerance* and maintained high tissue water status for longer. Significant level of *osmotic adjustment* (OA) was identified in pigeonpea, which benefited leaf survival. Pigeonpea invested significantly more total dry matter (TDM) in roots than either cowpea or soybean. Cowpea survived longest in pure stand whereas pigeonpea and soybean survived shortest in pure stand, suggesting that the dehydration avoidance response of cowpea was more effective in competition with like plants whereas the dehydration tolerance strategies of pigeonpea and soybean were least effective when competing against like plants. On the average, TDM/plant ranked in the order of cowpea > soybean > pigeonpea, largely reflecting initial differences in plant size when water was withheld. However, there was an inverse relation between TDM of a species and that of its competitor, so that in effect, water not used by a given plant to produce TDM was used by its competitor.

A large spectrum of genotype duration (from long to early and extra-early duration) and matching genotype duration with likely period of soil water availability is the first strategy used against terminal drought stress (Serraj *et al.* 2003). Drought resistant genotypes can avoid moisture stress through faster root growth. Onim (1983) observed the differences in root depth at 31 days between resistant and susceptible genotypes. The difference was up to 18 cm and deep rooting was positively correlated with seed yield/plant.

The effect of moisture stress imposed at pre-flowering stage has been associated with greatest reductions in nodule *nitrogenase* activity (70-90%), followed by the rate of photosynthesis (50-71%), and root and nodule respiration (31-45%). Large seeded cultivars have been observed more sensitive than small seeded ones. Among small seeded cultivars, those with indeterminate growth habit have been found more drought resistant than those with alternative type (determinate growth habit). Values for *relative water content* (RWC) and water retention in leaves were also higher in cultivars with indeterminate growth (Kuhad *et al.* 1989). Kimani *et al.* (1994) noted genetic variation for vegetative development, leaf water potential, RWC, photosynthesis, and stomatal conductance during two cycles of water stress and recovery. They suggested to use water status parameters (especially RWC) as indicators of drought tolerance while breeding for drought resistance in pigeonpea.

In short-duration pigeonpea, the maintenance of both leaf area index (LAI) and fractional canopy light interception appears to indicate genotypic drought tolerance. Some possible characteristics that may improve drought resistance of short-duration pigeonpea include the ability to maintain TDM, low flowering synchronization, small pod size with a few seeds/pod and large 100-seed weight (Lopez *et al*. 1996, 1997).

OA is considered as an important physiological mechanism of drought adaptation in many crop plants. In a group of 26 early-duration pigeonpea genotypes, Subbarao *et al*. (2000) observed a significant correlation of mean leaf osmotic potential with the mean OA under water stress condition at 60-92 days after sowing. Mean leaf osmotic potential accounted for 72% of the genotypic variation in OA. Significant genotypic variation was observed for the initiation, duration, and degree of OA. Genotypic differences in grain yield under drought was best explained using stepwise multiple regression to account for differences in OA at 72, 82, and 92 days after sowing ($r^2 = 0.41^{**}$; n= 78). The degree of OA at 72 and 82 days after sowing (DAS) contributed positively to the grain yield, whereas OA at 92 DAS contributed negatively to this relationship. In another study, Flower and Ludlow (1987) screened 22 pigeonpea accessions for variation in osmotic adjustment and dehydration tolerance of leaves. However, no difference in osmotic adjustment was found under field condition, but moderate variation (0.7-1.3 MPa) was found among twenty-two accessions grown under controlled conditions. In addition, moderate variation in dehydration tolerance was found among the accessions; lethal leaf water potentials ranged from -6.8 to -8.2 MPa, although the level of tolerance was high compared with other grain legumes. In view of the general genetic diversity of the accessions, they concluded that the probability of finding greater variation in these traits is small. Moreover, because pigeonpea has high OA and high dehydration tolerance compared to other crops, they suggested that high priority should *not* be given to attempting improvement of drought resistance by increasing the magnitude of these two traits in pigeonpea.

Screening of pigeonpea varieties for high reproductive fitness under actual water deficit condition may be a realistic approach. Reddy (2001) screened 'LRG 30', 'ICPL 85063', and 'ICPL 332' as the most suitable varieties out of the ten cultivars evaluated under rainfed condition. He concluded that higher yield of these cultivars (1980 kg/ha, 1800 kg/ha, 1787 kg/ha, respectively) under moisture deficit might be due to their high RWC, pods/plant and harvest index.

Jaleel *et al*. (2008) studied involvement of Paclabutrazol (PBZ) and ABA on drought induced *osmoregulation* in pigeonpea. They used two watering treatments (100 and 60% field capacity) to understand the effects of water deficit with and without PBZ and ABA on biochemical constituents, proline and antioxidant metabolisms of pigeonpea plants. There was a significant enhancement in the proline content and ã-glutamyl kinase and reduction in proline oxidase activities under water deficit stress. Drought stress caused an increase in the free amino acid and glycine betaine (GB) content. PBZ and ABA acted as stress ameliorating agents by further enhancing these parameters in water stressed pigeonpea plants. The stress mitigating effect was significant in the case of PBZ treated plants compared to ABA treated ones.

According to Kumar *et al.* (2011), a progressive water stress causes significant physiological and biochemical changes in pigeonpea. RWC parameter could be used to select high-yielding genotypes that maintain cell turgor under water deficit environment. Enhanced proline accumulation during stress indicated that proline plays a cardinal role as an osmo-regulatory solute in plants. The increased activities of antioxidant enzymes including superoxide dismutase (SOD) and peroxidase (POD) indicated that an effective antioxidant defense mechanism protects pigeonpea from destructive oxidative reactions.

In conclusion, physiological parameters such as dehydration tolerance, RWC and OA appear to be important in pigeonpea for combating moisture deficit condition. However, conclusive evidences that these parameters also confer reproductive advantages to the surviving genotypes are scanty. Agronomic traits such as pods/plant, seeds/pod, seed size and seed yield/plant under actual water deficit condition should be given much importance while breeding for drought resistance in pigeonpea.

C. Temperature Stress

In north India, pigeonpea experiences low temperature stress during winter months (December–January). The stress adversely affects growth, survival and reproductive capacity of plants if the minimum temperature falls below 5°C. At freezing temperature, intracellular water gets converted into ice, which in turn causes shrinkage of cells inside the plant, resulting in wilting and death of plants. According to Wery *et al*. (1993), the intracellular ice in the plants causes cell dehydration and cell membrane destruction due to freeze-thaw cycle leading to death of the plants under cold conditions.

Genetic variation for germinability and root-length under low temperature (14°C) has been noticed (Kumar *et al*. 1991). It has been established that greater root: shoot ratio (>1.0) may be used as a selection criterion for cold tolerance at the seedling stage (Kumar *et al*. 1991, 1995). In a field study in China, Yong *et al*. (2002) reported enormous variation in plant mortality and plant survival under cold stress condition (- 3°C to - 0.3°C). Conclusive evidence for the presence of genetic variability vis-à-vis cold tolerance was provided by Sandhu *et al*. (2007). They screened for cold tolerance in a set of 480 pigeonpea lines at PAU, Ludhiana. During the first fortnight of January, minimum temperature more often touches 0°C,

which was good enough to assess cold reaction. As many as 32 genotypes were rated cold tolerant as the plants retained their normal morphology with intact floral buds. They suggested for utilizing these genotypes to enhance cold tolerance of sensitive varieties and study the genetics of cold tolerance.

Upadhyaya *et al.* (2007) reported the results of an evaluation trial of pigeonpea germplasm accessions collected from low (<500 m), medium (501–1000 m), high (1001–1500 m) and very high elevation zones (>1500 m) of Kenya at ICRISAT, Patancheru, India. They observed that accessions from the very high elevation zone (>1500 m) were late flowering with a large number of tertiary branches, large seeds and a high shelling percentage and could be a source for cold tolerance and breeding of vegetable types. Results indicated that the elevation of collection sites is important in determining variation patterns of pigeonpea in Kenya.

Reports concerning effects of low temperature stress on the reproductive parts are only a few and also not well-documented. Singh *et al.* (1997) studied effects of low temperature on floral buds and flower drop in the pigeonpea germplasm. They identified seven highly tolerant genotypes (including 'Bahar', a leading variety of long-duration pigeonpea in northeastern plain zone of India) on the basis of low bud and flower drop. They observed that long-duration cultivars are well-adapted to cold situations because of their inherent genetic mechanism to cope up with very low temperature during reproductive stages. However, in both the years (1992-93 and 1993-94) during which observations were recorded, mean temperature did not fall below 14°C. Therefore, it does not seem convincing whether the variation in bud and flower drop was a consequence of low temperature stress. Choudhary (2007) recorded data on buds/plant and flowers/plant in two-temperature environments under field condition (mean temperature: 16.4°C and 11.4°C). Low temperature stress (11.4°C) appeared to reduce the number of buds and flowers in each genotype. 'IPA 7-2' (a selection from a local land race 'Kudrat-3') was identified as the most tolerant on the basis of least reduction and better mean performance for the number of buds and flowers under low temperature condition. However, the other genotype 'Bahar' also appeared at par with the 'IPA 7-2'. On the basis of rank correlation (0.943**), it was suggested that either of the two traits could be used as a selection criterion for tolerance to low temperature.

The research conducted at the IIPR, Kanpur (Annual Report 2008-09) revealed that low temperature primarily affects development and growth of flower buds. In some sensitive genotypes such as 'IPA 209' and 'IPA 06-1', filaments of stamens fail to enlarge at low temperature and thus affect opening of flowers. Pollen dehiscence does not occur too, although pollens are fully fertile. As a consequence, unfertilized flowers wither and fall down, resulting in no pod formation in these genotypes under low temperature. Detailed analyses of F_1, F_2 and backcross populations derived from crosses between sensitive parents ('IPA 209' and 'IPA 06-1') and tolerant parent ('Bahar') revealed that low temperature tolerance in pigeonpea is a monogenic dominant trait (Annual Report 2008-09), which could be easily transferred to high-yielding pigeonpea cultivars (such as 'NA 1') that are sensitive to low temperature.

In a similar study in another set of materials, Singh and Singh (2010) also substantiated the previous finding that pod setting in pigeonpea under low temperature is a dominant trait and is governed by a single gene. The lower limits of average minimum and maximum temperature during both the years (2003-04 and 2004-05) at the pod setting stage in the tolerant (such as 'MAL 19') parents, its crosses (such as 'NA 1' × 'MAL 19') with the sensitive (such as 'NA 1') parents and their segregating generations (F_2 and backcross generations) were 9°C and 22.1°C (mean temperature: 15.55°C), respectively. However, there are certain genotypes like 'IPA 209' and 'IPA 06-1' and 'Bahar' and 'IPA 7-2', whose degree of sensitivity and tolerance to low temperature are even greater than 'NA 1' and 'MAL 9', respectively.

All the above results related to the effects of low temperature stress on the reproductive traits of pigeonpea were carried out under field (uncontrolled temperature) conditions. Nonetheless, these studies threw at least some light on the number and nature of gene(s) that governs low temperature tolerance in pigeonpea. Screening of a large number of pigeonpea genotypes for low temperature tolerance under controlled temperature condition is still needed to confirm those findings and generate precise genetic information.

D. Photoperiod

Pigeonpea is known to be thermo- and photosensitive crop. It is grown in the areas where day length varies from 11 to 14 h and large differences in temperature are experienced, largely due to variations in altitude and latitude. Field studies have been conducted in pigeonpea with different maturity durations (extra-early, early, medium and long durations) in Kenya to determine the effect of photoperiod and temperature on flowering. It has been found that the extra-short duration genotype 'ICPL 90011' was the least responsive to variation in photoperiod, while the two long-duration genotypes 'ICEAP 00040' and 'T 7' were the most sensitive to photoperiod variation with flowering rate reduced by 0.001 d-1 per hour increase in day length (Silim *et al.* 2007). Carberry *et al.* (2001) found that flowering in short-duration pigeonpea cultivars was delayed by up to 100 days when day length in the photoperiod-inductive phase exceeded a critical value. Medium- and long-duration cultivars delayed flowering by over 150 days in response to photoperiod. A pioneer experiment was conducted by Hugh *et al.* (1985) to determine how the rate of development from sowing to flower bud

initiation (FBI) and sowing to flowering was affected by temperature and day length in pigeonpea cultivars with different maturity periods. They found that both temperature and day length had substantial effects over the range 16–32°C and 10–14 h, respectively and the rate of crop growth from sowing to flower bud initiation varied among cultivars. The effect of day length on the rate of crop growth was the greatest between sowing and flower bud initiation, with the greatest sensitivity between 12 and 14 h. For the range of conditions considered, temperature had at least as great an influence as day length on the rates of development from sowing to FBI and from sowing to flowering.

E. Soil Salinity Stress

Soil salinity can be a major constraint to pigeonpea in regions where it is predominantly grown (Subbarao *et al*. 1991). Salt accumulation in soil surfaces, known as soil salinity, could lead to the impairment of plant growth and development and is manifested mostly under irrigated and dryland agriculture. Excess salts in the soil affects plants through osmotic stress, accumulation to toxic levels within the cells, and through the interference with the uptake of mineral nutrients (Chikelu *et al*. 2007). Higher sodicity ($NaCl$ / Na_2SO_4) adversely affects the rate of photosynthate translocation from the source leaf to other plant parts including pods (Deshpandey and Nimbalkar 1982). It has been shown that higher concentrations of NaCl (15 m mhoes/cm^2) reduces plant height, leaf area, leaf area index (LAI), crop growth rate, net assimilation rate, total dry matter (TDM) production and seed yield and increases leaf thickness of pigeonpea (Joshi and Nimbalkar 1983). Salinity delays days to 50% flowering by 1-2 week and prolongs the peak period of flower production, and reduces number and weight of the pods and seeds (Promila and Kumar 1982).

There are reports related to field screening of pigeonpea for tolerance to soil salinity. Genetic variation for tolerance to salinity (with respect to survival) has been observed among cultivated genotypes and wild species especially *Cajanus scarabaeoides* (Rao *et al*. 1981). Some leading cultivars such as 'C 11' (Chauhan 1987), 'UPAS 120' (Promila and Kumar 1982), and the like have been identified as salinity tolerant.

Subbarao *et al*. (1991) studied comparative salinity tolerance among pigeonpea genotypes and their wild relatives. Among the cultivated genotypes, 'ICPL 227' and 'Hy3C' were observed as the most tolerant and the most sensitive genotypes, respectively. However, the extent of variation in salinity response among cultivated genotypes appeared too limited to warrant genetic enhancement of salinity tolerance. Among the wild relatives of pigeonpea, several species including *C. scarabaeoides, C. albicans* and *C. platycarpus* showed a wide range of variation in their salinity tolerance. The results suggested that using wild relatives for genetic improvement might increase salinity tolerance of pigeonpea. According to Subbarao *et al*. (1990), the transfer of salinity tolerance from *C. albicans* to *C. cajan* would be feasible as the high level of salinity tolerance in this wild species is expressed as a dominant genetic trait. They further clarified that certain physiological attributes that confer salinity tolerance in this wild species include *Na* and *Cl* retention in the roots and limited translocation to the shoots, high *K* selectivity and maintenance of transpiration rate under saline conditions.

In another experiment, Ashraf (1994) assessed salt tolerance of three pigeonpea accessions, namely Local arhar, 'ICPL 151' and 'ICPL 85014' at the germination, seedling and adult stages. There was no positive correlation between tolerance at the early growth stages and at the adult stage since no clear difference in salt tolerance of the three accessions was observed at the germination and the seedling stages, whereas accessions differed considerably at the adult stage. Although increasing salt concentrations adversely affected the growth of all three accessions, 'ICPL 151' was superior to the other two accessions in fresh and dry biomass, yield and yield components when tested at the adult stage. The tolerant accession 'ICPL 151' accumulated significantly lower $Na^+$and *Cl?* in shoots. By contrast, the accession had higher shoot and root K^+, *K/Na* ratio, *K vs. Na* selectivity, soluble sugars, free amino acids and proline compared to the other two accessions.

Differential tolerance to salinity vis-à-vis pigeonpea maturity groups has been observed (Dua and Sharma 1996). Late maturing genotypes showed better tolerance than early maturing ones. No correlation was found between the tolerance at germination and later stages. However, percentage survival showed some association with seed yield under salinity. Low and high accumulation of *Na* and *K*, respectively in the roots and other plant parts (main stem, branches and leaves) perhaps helped salinity tolerance in pigeonpea.

Efforts towards improvement of salinity tolerance through plant biotechnology have also been done. Several gene transfer approaches have been shown to improve the stress tolerance of the crop plants. The transferred genes include those encoding enzymes required for the biosynthesis of various *osmoprotectants*, or those encoding enzymes for modifying membrane lipids, LEA proteins and detoxification enzyme. Stress-inducible transcription factors have been demonstrated to have great potential (Sharma and Lavanya 2002).

Srivastava *et al*. (2006) found that a *NaCl* treatment of 1.01 g/kg alfisol was suitable to salinity screening in pigeonpea. Using that treatment, they found large variations in the salinity susceptibility index (SSI) and the percent relative reduction (RR %) in both cultivated and wild accessions. The amount of *Na* accumulation in shoot showed that more tolerant materials accumulated less *Na* in the shoot except the wild species, which followed a different pattern compared to cultivars. Overall, they found that *C. acutifolius, C.cajanifolius*

and *C.lineata* were mostly sensitive, whereas *C. platycarpus, C. scarabaeoides* and *C. sericeus* provided good sources of tolerance. It was interesting to notice that *C. scarabaeoides* also provided a large range of sensitive materials. It was expected that accessions originating from putative saline areas would provide higher levels of tolerance, but the minicore collection of pigeonpea provided a larger range of variation in the salinity response. It was noted that tolerant accessions may be obtained either from the minicore collections or from the set of accessions from putatively salinity affected areas. Besides, there was a large number of tolerant accessions originating from Bangladesh. Further work is going on to confirm these data to assess yield response to salinity and to develop intra-or inter-specific populations for the mapping of salinity tolerance.

Karajol and Naik (2011) assessed salinity tolerance among ten varieties of pigeonpea during germination at 0, 100, 125, 150, 175, 200 and 250 mM *NaCl* concentrations. Germination percentage was not much affected by salinity; however, it delayed germination at 250 mM in all accessions to varying degrees. The varieties with white seeds such as 'WRP 1', 'GS 1' and 'TS 3' appeared salinity tolerant compared to red or black seeded varieties like 'Black tur', 'Asha' and 'Bennur Local' accessions, which were rated highly sensitive to salt stress based on their germination rate and final germination percentage. However, they opined that final evaluation and selection for high-yielding tolerant genotypes in pigeonpea would require field evaluation in the salt affected soils. The superior performance of white seeded varieties over other varieties may be an interesting observation that needs further confirmation. If revalidated, it will form the basis for further improvements in the salinity tolerance of pigeonpea variety.

F. Aluminium Toxicity

To meet the growing demand of pigeonpea, it is imperative to expand its cultivation on wider scale in non-traditional areas such as hilly tracts of north eastern states. There are other states like Bihar, Jharkhand and Chhattishgarh where increasing trend in pigeonpea cultivation has been observed in recent years. However, these states have considerable acreage under acidic soils with the serious problem of aluminium (Al) toxicity (Choudhary and Singh 2011).

Most of the grain legumes including chickpea (Singh and Chaturvedi 2007), pigeonpea (Singh and Choudhary 2009, Choudhary *et al*. 2011), pea (Singh and Choudhary 2010) and alfalfa (Campbell *et al.* 1988) are sensitive to aluminium. Considerable variation for tolerance to aluminium toxicity in plant species and genotypes within species has been reported (Kinraide *et al.* 1985, Singh and Choudhary 2009). The work on screening for tolerance to Al toxicity in pigeonpea is only a few and also not well-documented.

The four techniques, which are commonly used for screening of Al toxicity in pigeonpea, are sand and hydroponic assays, hematoxylin staining and root re-growth assay (Singh and Choudhary 2009). These four techniques have unconditional advantages over field screening because reliable ranking of tolerance in the field screening is difficult due to the large temporal and spatial variation in acidic soils (Choudhary *et al*. 2011). Moreover, screening at field level is very expensive and time consuming when a large number of genotypes are under evaluation (Garcia *et al.* 1979). Besides, the results obtained with solution culture screening method correlate positively with those obtained using field screening (Urrea-Gomez *et al.* 1996), indicating reliability and dependability of laboratory screening methods.

Singh and Choudhary (2009) screened 32 genotypes of pigeonpea for tolerance to aluminum toxicity. No distinct and visible symptoms of aluminum toxicity were observed in the shoot of pigeonpea genotypes. However, restriction of root growth was observed. Shorter roots with absence of normal branching pattern were observed at higher levels of aluminum

Table 1. Some pigeonpea genotypes/ cultivars tolerant to abiotic stresses

Abiotic stress		Cultivars/genotypes	Tolerance mechanism	References
Moisture stress	Waterlogging	ICPL 84023 (early), Asha (medium)	Lenticels development, more root biomass and adventitious root	Sarode *et al*. (2007)
	Drought	LRG 30, ICPL 85063, ICPL 332 (medium)	High RWC, pods/plant and HI	Reddy (2001)
Temperature stress	Low temperature	IPA 7-2, Bahar, and MAL 19 (Late)	Ability to flower and pod setting under low temperature	Choudhary (2007), Singh *et al.* (1997), Singh and Singh (2010)
Mineral stress	Salinity	C11, ICPL 227, WRP1, GS1 and TS3 (medium) UPAS 120 and ICPL 151 (early)	Reduced translocation of Na and Cl from root to shoot	Chauhan (1987), Promila and Kumar (1982), Subbarao *et al.* (1991), Ashraf (1994) and Karajol and Naik (2011)
	Aluminium toxicity	IPA 7-10 and T 7 (late), 67 B and GT 101E (early)	Aluminium exclusion	Choudhary *et al.* (2011)

(30 and 50 µg/ml Al) compared to the control treatment (0 µg/ml Al).

The results of all the four methods (hydroponic and sand assays, hematoxylin staining and root re-growth methods) were almost similar, indicating that any one of these methods could be used to screen for aluminium tolerance in pigeonpea. These methods almost consistently discriminated between tolerant ('IPA 7-10', 'T 7' and '67 B') and sensitive ('Pusa 9', 'Bahar' and 'Pusa 2002-2') genotypes of pigeonpea at 30 or 50 µg/ml Al concentration (Choudhary *et al*. 2011). However, 30 ppm (µg/ml) was suggested as the optimum Al concentration to discriminate between tolerant and sensitive genotypes. The tolerant genotypes had greater root and shoot length, more root and shoot dry matter. These four parameters in both sand and hydroponic assays were highly correlated among themselves, indicating that any of them could be used (Choudhary *et al*. 2011). The intensity of hematoxylin stain for tolerant genotypes was scored as only *partial* compared to *complete* stain of sensitive genotypes. Root re-growth of all genotypes decreased significantly with an increase in aluminum concentration in nutrient solution. It virtually ceased in 'Bahar', 'Pusa 2002-2'and 'Pusa 9' at higher Al concentrations (30 or 50 µg/ml Al) due to irreversible damage caused to the root tips. Tolerant genotypes, namely 'IPA 7-10', 'T7', 'GT 101' and '67 B' had larger mean root re-growth (> 1.5 cm) than that of sensitive genotypes (0.25 cm) at 30 µg/ml Al concentration (Choudhary and Singh 2011).

Tolerant and sensitive genotypes were further assessed for phosphorus, potassium, calcium and magnesium contents in their root and shoot. Tolerant genotypes ('IPA 7-10', 'T 7', 'GT 101' and '67 B') accumulated significantly high amounts of these nutrients (> 1.5 times) compared to the sensitive ones in both root and shoot. Better performance of tolerant genotypes could be ascribed to better nutrient uptake efficiency and distribution within the plants (Choudhary and Singh 2011).

Aluminium concentration in the roots of both tolerant and sensitive genotypes was greater than that for the shoots. Root aluminium contents were significantly lower for the tolerant genotypes ('IPA 7-10', 'T 7') than for the sensitive genotypes ('Bahar' and 'Pusa 9') at both 20 and 50 ppm Al concentrations. This indicated that aluminium tolerance in these accessions of pigeonpea stemmed from aluminium exclusion from the root (Choudhary *et al*. 2011). In addition, shoot aluminium content was also considerably lower for the tolerant genotypes than for the sensitive genotypes. This could be ascribed to reduced translocation of aluminium from root to shoot in the tolerant genotypes. However, any sign of internal detoxification could not be detected.

Tolerant genotypes such as 'IPA 7-10', 'T 7', 'GT 101' and '67 B' may be used in future breeding programme to develop aluminium tolerant pigeonpea cultivars. However, further study involving land races and wild accessions (especially from *Cajanus scarabaeoides* and *C. platycarpus*) of pigeonpea for tolerance to aluminium toxicity under field condition (natural acid soil) is still required. This may generate comprehensive data for even higher degree of Al tolerance vis-à-vis reproductive parameters such as yield. This will also corroborate whether tolerance to Al toxicity in pigeonpea imparts only survival advantage or also confers increased reproductive fitness on the tolerant genotypes (Choudhary and Singh 2011).

G. Conclusions and Future Prospects

Available literature suggests that only limited efforts have been made to improve the abiotic stress tolerance in pigeonpea. Since the crop is largely cultivated in Indian sub-continent, greater efforts, of course, are expected from Indian scientists. Flow of funds should also come from Indian/ Asian governments. The current challenge in pigeonpea cultivation is to reduce the gap between potential and realised yield and to minimise yield differences among major pigeonpea growing zones in which above-mentioned abiotic stresses are prevalent. Efforts are required towards holistic management of these abiotic stresses through the development of resistant/tolerant cultivars with consistent performance across the environments. In this direction, a deeper understanding of the physiological and genetic bases of variation for tolerance to these stresses needs greater attention. As environment and G×E interaction accounts for more than 95% of the total variation in pigeonpea, more efforts are required towards the development of high-yielding stable cultivars. The narrow genetic base is a serious impediment to breeding progress in pigeonpea (Yang *et al*. 2006, also *see* Kumar *et al*. 2011 for details). As wild relatives are a rich reservoir of genes for resistance to biotic and abiotic stresses (Sharma *et al*. 2003), introgression of these genes is an option to genetically mitigate the effects of such stresses. Further, the exploitation of genomic tools in conjunction with conventional breeding programmes can also be helpful. In the past few years, a large number of genomic tools has been developed in pigeonpea for resistance to various stresses (Kumar *et al*. 2011). Further, intensive efforts through *in vitro* techniques are underway towards identifying complex abiotic stress traits, alien gene introgression aided by embryo rescue and rapid fixation of stress tolerant recombinants through doubled haploid breeding (Pratap *et al*. 2010). These techniques in combination with more efficient screening methods deserve special attention in the days ahead to make pigeonpea cultivation a promising, remunerative and viable option for pulse growing farmers of the world.

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