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Tailoring of Abiotic Stress Adaptive Traits to Diminish the Effect of Changing Climate on Crop Productivity

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ABSTRACT

The world population is likely to exceed 10 billion by 2050, thereby increasing food, feed and fuel production demand. On the other hand, global climate change (drought, heat, salinity, elevated CO_2 and extreme cold) hostile the global agricultural productions. The changes in climatic factors perhaps influence the crop distribution, affect the crop growth and yield, and increase the risks of farming and human health consequences in developing countries. Crop breeding is one of the approaches to fight environmental challenges in agriculture. Available literatures imply that genotypes of different crop species are expressing greater phenotypic variability to tolerate abiotic stresses by inherent constitutional. Hence, there is an opportunity for utilizing the existing variability in abiotic stress tolerance traits. The gene sources for abiotic tolerance are available in germplasm collection, landraces, or wild relatives, if not, with less frequency it can be created as transgenes, somoclones or mutants. However, to make significant advancement in abiotic stress breeding requires accurate and reproducible phenotyping under well-imposed stress environment. The targeted trait for abiotic stress tolerance should have high positive correlation with yield attributes and be amenable for scoring in given environment.

The traits introgressed for abiotic stress tolerance vary with stress scenario, timing and intensity of stress encountered by the crop species. Most of the traits that confer abiotic stress tolerance are quantitative in nature. The conventional crop improvement strategy followed to transfer abiotic stress tolerance is by recurrent selection and backcross breeding, which delivered limited success. The recent advancement through rapid and high-throughput phenotyping and genotyping have given much hope for tailoring desirable traits to evolve climate-resilient cultivars. The gene pyramiding strategy

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is useful to accumulate desirable abiotic tolerant traits into a commercially preferred cultivar. Further, transgenic and double haploid approaches will help in accelerating the trait pyramiding strategy. The climate-resilient cultivars with climate-smart farming will offer sustainable and cost-effective solution to the changing agro-climatic situations.

Keywords: Abiotic stress, breeding, phenotyping, tolerance, trait pyramiding

1. EXTENT AND IMPLICATIONS OF ABIOTIC STRESS IN AGRICULTURE

The global temperature over the last 100 years has increased by 0.74°C and is expected to increase by about 0.2°C per decade over next two decades. Global sea level has risen by 17 cm during the 20th century, in part because of the melting of snow and ice from many mountains and polar regions (Green Facts, 2007). Changes in temperature, rainfall pattern and increase in CO_2 levels will have drastic impact on global agriculture, especially on dryland ecosystems which supports 2.5 billion inhabitants (Anderson and Morton, 2008). In coming years, direct impact of climate changes in agriculture mainly on decreasing the water availability and soil moisture, increasing the ambient air temperature thereby increase land degradation and desertification. By the 2050s, 50% of agricultural lands are very likely to be subjected to desertification and salinization in some areas of Latin America. Yields from rain-fed crops could be halved by 2020 in some countries in Africa (UNFCCC, 2007). The climate changes influence the crop distribution, decline in crop growth and yield, and increase the risks that are associated with agriculture. At least 22% of the cultivated area under the world's most important crops is projected to face negative impacts from climate change by 2050 (Campbell *et al*., 2011). Abiotic stresses are the principal cause of crops failure worldwide which reduce the yield by more than 50%. A projected global impact of climate change by 2030 in agriculture for five major crops in each region is depicted in Figure 1. The major abiotic stresses that are expected to increase in response to climate change are heat, drought, salinity, waterlogging and inundation (Bray *et al*., 2000). These stresses are location specific, occurrence is unpredictable, degree of stress varies with crop season or year, one stress may increase or decrease the level of another stress for instance, in saline soil the moisture stress would enhance salinity stress (Singh, 2001). Experts say human population might exceed to 10 billion by 2050, in order to nourish the teeming millions, FAO estimated that the food production should be increased to 70% over the next four decades which is rearly impossible with current progress in densely populated developing countries like India. This implies that there is a pressure on agricultural scientists to evolve climatic resilient varieties and develop suitable management technologies to cope with abiotic anxieties. These hassles vary with timing, duration and intensity, thus sufficient knowledge of the target environment is essential in abiotic stress tolerance breeding. Hence, it is time to look for suitable crop hybridization strategies to mitigate various kinds of stresses and to ensure food security for teeming millions.

2. DROUGHT STRESS

Agricultural drought results in reduction of crop yield due to shortage of soil moisture availability in the root zone (especially on rain-fed or dryland conditions) and it is highly variable in its timing, duration and severity, which results in high environmental variation and genotype × environment interaction. The impact of drought stress in rice is depicted in Figure 2 using the data from Boonjung and Fukai (1996), which shows 20% yield loss at maturity. Similarly, groundnut germplasm were screened during rainy and post-rainy seasons, post-rainy crop suffered with soil moisture stress to an extent of 50 per cent during flowering and pod development stages. The soil moisture stress in peanut delayed the flowering and maturity (Figure 3), and showed negative influence on plant height, number of pods per plant and pod yield per plant (Arunachalam *et al*. 2012).

The drought resistance cultivar has the ability to withstand or minimize the yield loss due to moisture stress by various mechanisms, *viz*., drought escape, dehydration avoidance and dehydration tolerance. The dehydration avoidance genotypes can be further viewed as 'water savers' by reduced transpiration and 'water spender' by increased water uptake, these were well described in many literatures. Developing of crop varieties with low-water requirements and better water-use efficiency is essential to minimize the total production loss.

2.1 Managing Drought Environment for Screening

The plant traits associated with drought avoidance and tolerance can be *constitutive* that differs between genotypes, or *adaptive* which vary with the stage of the life cycle. In plants, constitutive traits are inherent to the genotypes like early flowering, rooting patterns, water use efficiency, epicuticular wax, stomatal index and phenology — which are not under control of stress responsive genes and do not require drought conditions for selection. Acquired traits/mechanism are upregulated under stress such as osmotic adjustment, changes in abscisic acid (ABA), tolerance to oxidative stress and reserve mobilization to the grain (partially stress responsive). Such drought responsive and adaptive traits requires specific stress environment to express in plants (Blum, 2011). Stress environment is apparent to identify the stress tolerant genotype or to score the desired trait. Hence, drought-screening fields require a careful management to avoid epigenetic variation among plots. To identify sources of drought resistance the screening methods should be simple and reproducible under the target environment.

2.1.1 Moisture Stress Environment in Field Conditions

The genotypes can be exposed to varied moisture stress levels in the field condition by the following ways.

(i) *Artificially create stress in the "normal" growing season:* Conducting experiment in normal growing season helps to avoid genotype x season problems. The varied moisture regime during particular crop growth stage can be achieved by (a) providing controlled irrigation in the field; (b) line-source-sprinkler irrigation method used to create a gradient of drought stress and help to evaluate large numbers of genotypes at varying intensity of drought (Serraj *et al*. 2003), the statistical parameter to assess the sensitivity of genotype to drought was dealt by Singh *et al*. (1991); and (c) drip irrigation system also can be designed to deliver the desired volume of water and manage the stress levels for wide spaced and row crops. It is important to eliminate the undesirable rainfall on the experimental field by rain-out shelter and is a costly affair.

(ii) *Evaluating in 'desert' environment:* It is ideal to carry out the phenotyping for drought tolerance in the 'desert' environment, where no unexpected rainfall can be interfere with the managed stress. Breeder can have total control of the moisture regime through irrigation (Blum, 2011). But, the phenotyping field should be surrounded by sufficiently wide crop-border to avoid the 'fetch' or dry wind effect in desert.

(iii) *Managing water-deficits in a non-growing season:* To avoid unpredictable seasonal rainfall during phenotyping, the drought screening experiments are planned in offseason or dry environment. But it needs application of uniform moisture stress environment. In this method, there is a possibility to maximize genetic component of the observed variation. But the extrapolation of results to 'natural' target environment or normal growing season can be difficult.

(iv) *Delayed Planting:* The delayed planting was done such a way that crop exposed to terminal drought. This makes amenable for phenotyping the flowering traits and grain development under moisture stress and selecting the genotypes for terminal stress tolerance.

2.1.2 Managed Moisture Stress Environment in Pot Culture

Imposition of uniform drought stress under field conditions at every plot is difficult. On the other hand, pot culture studies serves better managed stress environments. Different methodologies have been used to stimulate the moisture stress to known quantum at specific stages at whole plant level in pot experiments. An ideal technique used for the simulation of drought stress in pot studies should (a) maintain uniform moisture content within the root zone, (b) allow the rate at which stress develops to be precisely controlled, (c) permit a range of physiologically distinct stress levels to be imposed and maintained indefinitely, and (d) provide a mean of accurately quantifying stress level. The stress can be imposed for pot studies by gravimetric method or computer automated null-balance lysimeter as described by Earl (2003).

2.2 Drought Tolerant Traits and their Phenotyping

Phenotyping is the comprehensive measurement of individual quantitative parameter that forms the basis for the complex traits such as plant growth and development, plant architecture, yield, and physiological mechanism under stress environment. The traits underlying the different mechanism by which the plant mitigates the drought and ways to phenotype those traits are discussed below.

2.2.1 Drought Escape

Genotypes having rapid growth, early flowering and maturation are preferred to avoid the terminal drought. This can be easily scored by assessing day to flowering and maturity.

2.2.2 Dehydration Avoidance

In case of dehydration avoidance, the changes in the anatomical and morphological traits help the plant to increase water uptake and reduce water spending. The water uptake is increased by extensive root system with large active surface area and shoot/root ratio shifted in favour of the roots. The water loss is reduced by reducing transpiration through timely stomatal closure, thick cuticle, epicuticular waxes, white hairs on leaves, leaf angle, leaf rolling and plant senescence. The screening traits used to assess dehydration avoidance response of genotypes and their relevance, and ways to measure the trait of interest is discussed below:

(i) *Dehydration symptom in leaves:* It can be measured by scoring of leaf rolling or per cent reduction in leaf width resulting from rolling. Based on wilting symptoms the slow witling or delayed leaf rolling genotypes under water deficit conditions can be identified for the drought tolerance.

(ii) *Plant water status:* The total plant water potential is measured by the pressure chamber technique and thermocouple psychrometer method. The relative leaf water content (RLWC) can be measured at flowering and grain or pod development stage. The canopy temperature (CT) variation among the genotypes can be measured by infrared thermometer (IRT). It is an efficient method for rapid, non-destructive monitoring of whole-plant response to water stress. The genotypes relatively lower canopy temperature should be given importance in selecting to drought tolerance.

(iii) *Osmotic adjustment:* As a plant detects a water-deficit, it may accumulate a variety of osmotically active compounds such as amino acids, sugars and ions inside its cells, resulting in a lowering of the cell osmotic potential. Then the water present in intercellular spaces flows towards the inside of those cells. This process is called "osmotic adjustment" (OA). The amino acid, proline accumulation was observed significantly more in tolerant rice genotype under aerobic condition (Sritharan and Vijayalakshmi, 2007). Osmometer is used to measure osmotic potential. The leaf water potential is measured by pressure bomb apparatus.

(iv) *Stomatal activity and transpiration:* Leaf transpiration partitioned into stomatal and non-stomatal (cuticular) transpiration. Stomatal transpiration or conductance can be assessed by porometer. It is measured on a fully expanded young leaf. The cuticular transpiration can be phenotyped by two approaches (a) quantifying epicuticular wax load by colorimetric assay (Ebercon *et al*., 1977), and (b) assessing leaf water loss by diffusion porometer and weight of detached leaves over specific time interval under standard conditions. Genotypes with a thick cuticle layer retain their leaf turgor for longer periods of time after the onset of a water-stress. The drought tolerant rice genotype exhibited better epicuticular wax content under aerobic condition (Sritharan and Vijayalakshmi, 2007).

(v) *Non-senescence (stay-green):* The non-senescence or the chlorophyll retention parameter under stress condition is an important indicator to identify the drought tolerant types. Stay green trait can be assessed by visual scoring, measuring the per cent green leaf area and SPAD meter reading. The stay-green type is also judged by (a) estimation of normalized difference vegetation index (NDVI) at physiological maturity; and (b) the rate of senescence (RS). Lopes and Reynolds (2012) suggested a simple and integrated way to measure stay-green in large sets of germplasm using a "GreenSeeker" sensor to measure NDVI during the grain-filling stage in wheat. The precision of estimation will increase with the number of NDVI measurements taken and, probably after mid-grain filling; two weekly measurements should be taken under stressed environments.

(vi) *Measuring roots:* In abiotic stress breeding the root phenotyping is play an important role. In spite of number of constrains, the out-of-field methods are widely used for root phenotyping. The various versions of the containers like upright (split) PVC tubes, large polycarbonate boxes, big pots or containers and temporary rhizo-structures to mimic field conditions are used to grow the plants. The plants are removed without damaging roots, and the root traits, namely, root length, root length density, root volume is studied. The root parameters can also be measured by minirhizotron image analysis by non-destructive means in the field. The hydroponics also used to study the root architecture. The root function can be indirectly phenotyped by assessing plant water status in a drought managed conditions (Blum, 2011). Root penetration capacity is an important factor in avoiding drought where soil hindrances exist. To assess the root penetration capacity the root penetration of a wax layer method is widely used.

(vii) *Leaf anatomy:* The traits like waxiness, pubescence, rolling, thickness, leaf angle and leaf movement help to decrease radiation load on the leaf surface when the plant is in moisture stress. Which lower evapotranspiration rate and reduced risk of irreversible photo-inhibition. However, they may also reduce radiation use efficiency, in turn, reduce yield under favorable conditions.

2.2.3 Dehydration Tolerance

It is the ability of the plant as a whole or in any of its components to function under low plant water status (Blum, 2011). The dehydration tolerance ability of the genotypes can be assessed by following parameters.

(i) *Survival and phenology:* Seedling survival or recovery is a useful index of dehydration tolerance. This can be assayed by the 'box' test or seedling wilting test (Blum, 2011). Delayed heading or flowering, short anthesis-to-silking interval (ASI) in maize, scoring fertility and seed set can be used as selection index. Photosynthetic rate of genotypes can be measured using portable photosynthesis system under stress and non-stress conditions.

(ii) *Cell membrane stability (CMS):* It is the ability of the cell to sustain cell activity and survive under dehydration or reduced water content or high temperature. Otherwise it is the capacity of the cell membrane to prevent electrolyte leakage at decreasing water content. The tolerant rice genotype showed better CMS under aerobic condition than other tested genotypes (Sritharan and Vijayalakshmi, 2008). Measurements of CMS reported to be correlated with yields under drought/temperature stress in different crops. CMS is estimated by subjecting leaf disc to stress and measuring the leakage of solutes into deionised water conductometrically.

(iii) *Partitioning and stem reserve mobilization for grain filling:* As photosynthesis becomes inhibited by moisture or heat stress, the grain filling process becomes increasingly dependent on stem reserve utilization. So the grain number maintenance, grain filling duration and grain filling index can be accounted to asses this trait under stress and non-stress conditions. Blum *et al*. (1983) proposed the use of chemical desiccation of the canopy after flowering as means for inhibiting current plant photosynthesis. Chemical desiccation of plant canopies at the onset of grain filling was developed as a tool for revealing genotypic differences in grain filling from stem reserves in the absence of current photosynthesis (Blum *et al.,* 1983a). Potassium iodide (KI) is a chemical contact-desiccant was used for assessing genotypic diversity in grain filling under drought stress (Tyagi *et al*., 2000).

(iv) *Plant growth and productivity*:

(a) **Plant growth attributes:** The key indices assessed to understand growth responses are relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), specific leaf area (SLA) and leaf weight ratio (LWR).

(b) **Yield parameters:** The yield response of genotypes to moisture stress can be assessed by the different drought tolerance indices such as mean productivity (MP) under stress and non-stress conditions (Rosielle and Hamblin, 1981), drought susceptibility index (DSI) (Fischer and Maurer, 1978), drought tolerance efficiency (DTE%) (Fischer and Wood, 1981) and stress tolerance index (STI) (Fernandez 1992) and harvest index (HI). The genotypes with high value of MP, DTE, STI and value below 1 of DSI were considered drought tolerant genotypes.

(c) **Water use efficiency:** Water use efficiency (WUE) can be determined gravimetrically as per Udayakumar *et al*., (1998) or by using a computer automated gravimetirc lysimeter in association with physiological parameters. The carbon isotope discrimination (CID) technique is also used to determine the WUE; but it needs $\delta^{13}C$ analysis by isotope ratio mass spectrometer (IRMS). Alternate strategies may be adopted to measure the WUE using the surrogate traits such as SLA, SCMR and stomatal index. SLA is the ratio of leaf area to leaf dry weight. It is an indirect measure of leaf expansion. Higher SLA represents larger surface area for transpiration and hence SLA and WUE would be inversely correlated (Karaba *et al*., 2011). Sritharan and Vijayalakshmi (2010) reported that $\delta^{13}C$ is negatively correlated with WUE in rice genotypes under aerobic condition. However, it had positive correlation with total drymatter production, leaf rolling, chlorophyll stability index, relative water content, total chlorophyll content, soluble protein and nitrate reductase activity (Sritharan and Vijayalakshmi, 2012). Stomatal index (SI) can be computed by impression method to count the stomata under microscope. SPAD chlorophyll meter reading (SCMR) is a non-destructive method of measuring chlorophyll content in the leaves by hand-held chlorophyll meter. It determines the relative amount of chlorophyll present in the leaf. The SPAD value is measured on a fully expanded young leaf of the plant.

v. Hormonal regulation: The abscisic acid (ABA) is known as *stress hormone* and its concentration increases under moisture stress in plants. The ABA-mediated stomatal closure is a known mechanism under drought to reduce the water loss by leaf transpiration, reduction in leaf expansion and promotion of root growth. On the other hand, ABA has negative effect on plant during flowering by decreasing the pollen viability and seed set.

3. SALT STRESS

The climate change causes melting of glaciers can threaten mountain settlements, water resources and increase the damage associated with coastal flooding. Global average sea level is expected to rise by 18 to 59 cm by the end of the 21st century (GreenFacts, 2007). This leads increased salinization of arable land is expected to have devastating global effects, resulting in 30% land loss within next 25 years and up to 50% by the middle of 21st century (Wang *et al*., 2003). Agricultural land and thus food security affected by sea level rise, inundation, soil salinization, seawater intrusion into freshwater lenses (UNFCCC, 2007). To cope with this situation, it is essential to evolve saline tolerant cultivars to sustain crop yield in these areas.

3.1 Salt Stress Effects on Plant

Salts taken up by plant influence the plant growth by affecting turgor, photosynthesis or enzymes. Buildup of salt in old leaves accelerates their death, and loss of these leaves affects the supply of assimilates or hormones to their growing regions and thereby affect plant growth (Munns, 1993). The deleterious effect of salt stress on plants is physiologically lowering of osmotic pressure by decrease osmoprotectants and lowering antioxidants. Salinity affects the germination, vigour, root and shoot length; morphologically stunted plant growth, chlorosis, wilted and senescence, and arrest of plant growth leads to plant death. Plants are classified as glycophytes or halophytes according to their ability to grow on high salt environment. Most plants are glycophytes and cannot tolerate salt stress due to the following reasons:

- Soil salinity decreases the osmotic potential of soil solution creating water stress in plants and affects plant growth and development. High concentrations of Na^+ disturb osmotic balance and results in "physiological drought", preventing plant water uptake. Whereas, halophytic plants tolerate sodium toxicity, osmotic stress might be the main reason of growth inhibition (Türkana and Demiral, 2009). Many glycophytes respond to relatively low salt concentrations (< 6,000 mg/L, or roughly 100 mM) by "salt exclusion," particularly through low rates of net transport of sodium or chloride, or both, from root to shoot. Most of these salt-excluding glycophytes cannot adjust osmotically to the low external water potential by increased synthesis of organic solutes and, therefore, suffer from a decrease in turgor. Hence, salinity may induce an osmotic stress in glycophyte (Lauchi and Epstein, 1984).
- Infux of Na^+ dissipates the membrane potential and facilitates the uptake of Cl^- down the chemical gradient, which disrupts cell ionic equilibrium (Mahajan and Tureja, 2005). This leads to severe ion toxicity to cell metabolism and cause deleterious effect on some enzymes, since Na^+ is not readily sequestered into vacuoles in glycophytes as in halophytes. High Na^+ levels also lead to reduction in photosynthesis and production of reactive oxygen species (Flowers *et al*., 1977; Greenway and Munns, 1980; Yeo, 1998).
- The interactions of salts with mineral nutrition may result in nutrient imbalances and deficiencies in crop plants.

3.2 Salt Stress Tolerance Mechanism

The salt-resistant glycophyte can adjust osmotically to a saline medium. Salinity stress is first sensed in the root, but osmotic adjustment as well as growth inhibition and ion toxicity are most apparent in the shoot. Thus, in addition to cellular processes, root-shoot interactions and the coordination of the whole plant are an integral part of the responses to salinity (Lauchi and Epstein, 1984). Mechanisms for salt tolerance are of two main types: those minimizing the entry of salt into the plant, and those minimizing the concentration of salt in the cytoplasm (Munns, 2002).

To overcome the detrimental effects of salt stress, plants have evolved many biochemical strategies like (i) selective build-up or exclusion of salt ions, (ii) control of ion uptake by roots and transport into leaves, (iii) ion compartmentalization, (iv) synthesis of compatible osmolytes, (v) alteration in photosynthetic pathway, (vi) changes in membrane structure, (vii) induction of antioxidative enzymes and (viii) stimulation of phytohormones (Parida and Das, 2005).

In order to prevent water loss from the cell and to protect the cellular proteins, plants accumulate many metabolites with an osmolyte function such as sugars, mainly fructose and sucrose, sugar alcohols and complex sugars like trehalose and fructans. Since water moves from high water potential to low water potential, the accumulation of osmolytes make the water potential low inside the cell and prevent the intracellular water loss (Mahajan and Tureja, 2005). Tavakkoli and coworkers (2012) suggested that salt exclusion coupled with a synthesis of organic solutes is an important component of salt tolerance in the tolerant genotypes. The halophyte *Mesembryanthemum crystallinum* has emerged as a model system for understanding the molecular response to salt stress. This plant switches from C3 photosynthesis to crassulacean acid metabolism (CAM) in response to salt or drought stress (Sairam and Tyagi, 2004).

Salinity stress response is multigenic, as a number of processes involved in the tolerance mechanism, such as various compatible solutes/osmolytes, polyamines, reactive oxygen species and antioxidant defence mechanism, ion transport and compartmentalization of injurious ions (Sairam and Tyagi, 2004). Salinity tolerance was defined as the genotypic differences in biomass production in saline versus non-saline conditions over prolonged periods, of 3–4 weeks (Munns and James, 2003).The mechanisms of salt tolerance are categorized as (a) tolerance to osmotic stress, (b) Na^+ exclusion from leaf blades, and (c) tissue tolerance and the detailed are discussed by Munns and Tester (2008).

3.3 Salt Stress Imposition in Plants

The screening for salt tolerance requires reliable and controlled methods to maintain the salinity stress levels; and precise quick techniques to measure the salinity tolerance of plants. The saline environment can be created by adding salt (NaCl) solution to the growing media or soil. The nutrient contents should be sufficiently maintained to avoid the interference of nutrient deficiency during the salt stress screening either in field or in pots. The *in vitro* and *in vivo* techniques used to create varied salt stress environment for the screening programme is described below:

- ***In vitro* methods:** The techniques like filter papers with saline solution, salt-containing agar plants are used to assess tolerance potential of germination and seedling traits. In pot grown plants, the salt concentration is increased gradually by adding saline water in short time intervals for plant to adjust. Then periodically pots to be watered with saline solution to maintain the different levels of salt stress. The *in vitro* protocol for salinity screening in rice using seedling float for seedling, vegetative and reproductive stages is described in detail by Gregorio *et al*. (1997). Hydropnic cultures can also be used for salt stress evaluation purpose.
- **Field screening:** To create and maintain the salt stress level in field condition, non-saline field is irrigated with known quantum and concentration of saline solution. This strategy is used to assess the response of the genotypes to salt stress under field environment.

- **Screening in micro-plots:** It is a series of dug-out cavity structures made of brick-mortar-concrete materials and filled with artificially prepared or transported problem soil. It helps to mimic field environments and to maintain the varying levels of controlled salinity and sodicity. This technique is useful to avoid the soil heterogeneity and spatial variability in field that affect the reliability of genotypes response.

3.4 Phenotyping for Salinity Tolerance

Salt tolerant plants differ from salt-sensitive ones in having a low rate of Na^+ and Cl^- transport to leaves, and the ability to compartmentalize these ions in vacuoles to prevent their build-up in cytoplasm or cell walls and thus avoid salt toxicity (Munns, 2002). The genotypes with salt exclusion property need not to be a high yielder. If the osmotic effect of the salinity is dominated in salt tolerant lines it may affect the yield potential. Measurements of germination, survival, growth, and leaf gas exchange will indicate the osmotic effect of salinity. If the experiments are conducted for lengthy periods of time, measurements of growth, survival and gas exchange will also reflect toxic effects of salts in the leaves. An ideal high yielding salt tolerant variety should have good initial vigour, low Cl^- uptake, low Na^+/ K^+ ratio, good Na^+ excluder either in shoot or leaf, high tissue tolerance and agronomically superior characteristics with high yield potential. The selection criteria are to assess salt tolerance in plants is narrated below:

(i) Measuring cell survival, germination and survival of the plant under salt stress.

(ii) Leaf injury/death or senescence: Measuring the electrolyte leakage from leaf discs, chlorophyll content by SPAD meter and chlorophyll fluorescence. Decrease in chlorophyll content with increase in salinity level was observed in rice genotypes. The tolerant genotypes recorded higher chlorophyll stability index (CSI) than susceptible cultivars (Sritharan and Mallika Vanangamudi, 2006).

(iii) The parameters like Na/K ratio, Na and K uptake can be estimated to assess the salt tolerance of the genotypes. It was reported that the tolerance of a genotypes in rice have ability to restrict toxic ion uptake like Na^+ and have preferential uptake of the balancing ion like K^+. Lower Na/K ratio is considered desirable trait as it maintains the ion balance.

(iv) Tissue tolerance is measured in terms of LC_{50}. It is the concentration of sodium in the leaf tissue which causes a 50% loss of chlorophyll (Yeo and Flowers, 1983).

(v) The damage to chlorophyll apparatus can be assessed by measuring chlorophyll fluorescence by chlorophyll fluorometer.

(vi) The effect on salt stress on plant growth and yield can be measured by root elongation, leaf elongation, dry matter accumulation or biomass production and yield in saline environment over non-stress condition.

While screening for the salt tolerance, it is important to consider the concept of a two-phase growth response to salinity as suggested by Munns (1993).

- **Phase 1 (osmotic stress):** In this phase, visible quick reduction in growth is due to the salts outside the roots. It is essentially a water stress or osmotic phase, for which little genotypic variation among the genotypes. The growth reduction is presumably regulated by hormonal signals coming from the roots.

- **Phase 2 (salt-specific effect):** There is a second phase of growth reduction, which takes time to develop, and results from internal injury. It is due to salts accumulating in transpiring leaves to excessive levels, exceeding the ability of the cells to compartmentalize salts in the vacuole. This will inhibit growth of the younger leaves by reducing the supply of carbohydrates to the growing cells.

4. HEAT STRESS

The earth's climate is predicted to warm by an increase in mean ambient temperatures between 1.8 and 5.8°C by the end of the 21st century mainly due to both anthropogenic and natural factors. Future climates will also be affected by greater variability in temperature and increased frequency of hot days (IPCC 2007). Heat stress is often defined as the rise in temperature beyond a threshold level for a period of time sufficient to cause irreversible damage to plant growth and development. In general, a transient elevation in temperature, usually 10 to15°C above ambient, is considered heat shock or heat stress. Heat stress, singly or in combination with drought, is a common constraint during anthesis and grain filling stages in many cereal crops of temperate regions (Wahid *et al*., 2007).

4.1 Response of Plants to Heat Stress

In response to high temperature the plants manifest different mechanisms, including long-term evolutionary phonological and morphological adaptations and short-term avoidance or acclimation mechanisms. The crop plants tend to reduce heat-induced damage by phonological and adaptation changes. The adaptation mechanism varies with the crop stage at which exposed to heat stress. The leaf rolling, leaf shedding, reducing leaf size, thickening leaves, reducing growth duration, transpirational cooling or alteration of membrane lipid compositions and other adjustments in morphology and ontogeny mechanism help plants to overcome the heat stress (Wahid *et al*., 2007).

Leaf senescence starts early in response to heat stress, particularly when stress occurs during post-flowering stages of grain filling stages. Poor fruit/grain set at high temperature cannot be attributed to a single factor; decreases in pollen germination and/or pollen tube growth are among the most commonly reported factors. Therefore, pollen viability has been suggested as an additional indirect selection criterion for heat tolerance (Wahid *et al*. 2007). Grain development is impacted by heat stress because assimilate translocation and grain-filling duration and rate are influenced directly by changes in ambient temperature. The extent of heat-driven damage is dependent on the level of heat stress (Farooq *et al*., 2011). The maintenance of leaf chlorophyll and photosynthetic capacity at maturity, called 'stay-green', is considered an indicator of heat tolerance (Fokar *et al*., 1998).

The heat-shock response is a reaction caused by exposure of an organism's tissue or cells to sudden high temperature stress, and it is characterized by a transient expression of heat-shock proteins (HSPs). In plants following temperature elevation, the heat signal is probably transduced by several pathways that will, however, coalesce into the final activation of heat-shock transcription factors (HSFs), the expression of HSPs and the onset of cellular thermo-tolerance (Saidi *et al*., 2011).

Heat resistance is defined as the ability of a cultivar to yield more than other cultivars when subjected to heat. The plant overcomes the high temperature by heat avoidance and heat tolerance mechanisms. *Heat avoidance* is the ability of the genotype to dissipate the radiation energy to avoid rise in plant temperature. The plant reduces the heat by the process of transpiration cooling, leaf reflectance to reduce heat by pubescence and glaucousness. *Heat tolerance* is the ability grow and produce economic yield under high temperatures by improving membrane stability, reduced heat sensitivity to photosystem II, photosynthate translocation, stem-reserve mobilization and osmoregulation.

4.2 Selection Environment to Heat Stress

The selection environments for heat stress can be of *in vitro* or field environment (Singh, 2001), and moisture stress to plants should be avoided during screening for heat stress.

(i) *Screening under 'abnormal' field environment:* The screening at the location having high temperature or during off-season (summer).

(ii) *Managed environments:* The plant growth chambers can be used to manage the desirable level of heat stress during the specific period of plant growth.

(iii) *In vitro methods:* Screening of genotypes for high temperature stress in natural conditions, which are highly variable and is very difficult. The best alternative is to follow suitable laboratory procedures for screening. Temperature induction response (TIR) is a screening protocol developed based on the principle of "acquired tolerance" in which exposure of seedlings to a sub-lethal level of specific stress is used to induce tolerance to a subsequent lethal level of stress. By adapting this technique the genotypes were screened for thermo-tolerance (Kheir *et al*., 2012). TIR techniques wherein the genetic variability for temperature stress response can be examined in crop plants by assessing the survival, recovery and growth rates after exposing the germinated seedlings to lethal temperature stress. TIR is a robust and powerful technique and can be used to screen breeding lines or germplasms to identify thermo-tolerant lines. The usefulness of TIR technique was demonstrated in groundnut and cotton to assess the response of genetic lines to heat stress (Gangappa *et al*., 2006; Kheir *et al*., 2012).

4.3 Phenotyping for Heat Stress

Based on the adaptation mechanism expressed by the crop plants at different stages to heat stress, the selection of suitable traits for screening programme is vital to breeding for heat tolerance. The physiological and morphological traits have been evaluated for heat tolerance in plants are membrane thermo-stability, canopy temperature, leaf chlorophyll, leaf conductance, higher photosynthetic rates, stay-green, spike number, biomass, and flowering. Dias and Lidon (2009) proposed that high grain-filling rate and high potential grain weight as useful selection criteria for improving heat tolerance. The indices have been developed to measure the thermo-tolerance in crops based on the comparison of midday foliage, air temperature and their differential (Singh *et al*., 2007). They are canopy temperature depression (CTD), crop water stress index (CWSI) and thermal stress index (TSI). To screen for leaf tolerance to heat in breeding programme the canopy temperature depression (CTD) and leaf conductance can be effectively used for early generation selection (Reynolds *et al*., 2001). The screening traits used to assess heat tolerance and ways to quantify the trait of interest is discussed below:

(i) Germination, seedling mortality and recovery growth, canopy temperature depression and cellular membrane thermo-stability (CMT).

(ii) *Leaf conductance:* Leaf diffusive resistance was linearly related to leaf water potential (O'Toole and Moya, 1978). The stomatal conductance/resistance can be measured by steady state porometer or portable photosynthesis system.

(iii) *Chlorophyll content and fluorescence:* Heat stress reduces photosynthesis through disruptions in the structure and function of chloroplasts, and reductions in chlorophyll content (Xu *et al.,*1995). Photosystem II and chloroplast are sensitive to heat leads to damage to PSII in turn reduce the photosynthesis. Chlorophyll content meter (CCM) or SPAD meter can be used to assess the leaf chlorophyll content. The use of chlorophyll fluorescence as a screening trait, given that association between plant tolerance and lower fluorescence signals have been reported in a number of crops. Chlorophyll fluorescence can be measured through chlorophyll fluorometer.

(iv) *Pollen viability, fruit set and grain filling:* The heat sensitivity of genotypes in reproductive phase can be assessed by flowering, pollen sterility and seed set. The high grain-filling rate and high grain weight are useful selection criteria for improving heat tolerance.

(v) *Growth and yield:* The biomass, yield and harvest index under heat stress are used as selection criteria for heat tolerance.

5. SCREENING AND BREEDING FOR CLIMATE-RESILIENT VARIETIES

The crop improvement strategies, followed to evolve abiotic stress resistant varieties, are direct and indirect. In direct breeding, the genetically diverse materials are raised under stress environment and selection is done based on survival, yield and traits contributing to stress resistance. Whereas, in indirect approach the breeding materials are not deliberately developed for stress tolerance, but they are evaluated for stress resistance under specified environmental condition after it has been developed (Singh, 2001).

Importance of screening and phenotyping: The abiotic stresses are rarely singular, more likely they impart on plant populations as a set of interacting multiple stresses present in the given environment (Jarvis *et al*., 2005). The plants immobility limits the range of their behavioural responses to environmental signals and places a strong emphasis on cellular and physiological mechanisms of adaptation and protection to abiotic stresses (Wahid *et al*., 2007). The genotypes of different crop species express greater phenotypic variability in their ability to tolerate/resist abiotic stresses or in different environment.

Hence, it is impossible to make significant advancement in crop improvement without accurate and reproducible phenotyping under mutable stress environment (McClean *et al*., 2011). The rapid advancement can be achieved in abiotic stress tolerance by screening and selecting breeding materials in the target environment rather than doing in controlled conditions. The stress tolerant traits are the plant characters or a biochemical mechanism that facilitates cell survival and plant growth under stress condition. The screening trait for the abiotic stress should have desirable characteristics (Serraj *et al*., 2003) of strong link with higher or more stable grain yield in target environment, high level of heritability and easily measurable expression of tolerance is highly preferred.

A major challenge in traditional breeding for abiotic stress tolerance is the identification of reliable screening methods and effective selection criteria to facilitate detection of abiotic tolerant traits in plants under controlled stress environment. Controlled stress environment means imposing stress to appropriate duration and severity of stress at the desired plant growth stage. The phenotyping of abiotic stress tolerance for drought, heat and salt stress is described in the respective sections.

Care to be taken for managing stress environment in field conditions: The site homogeneity and control over the water regime or stress level are two important factors that determine the success or failure of managing stress environment in field conditions (Blum, 2011).

(i) *Site homogeneity:* It is most crucial in phenotyping of genotypes under field conditions. The important generators of site variability are (a) topography, (b) soil variability such as soil pH, and mineral deficiency, (c) bordering trees and their root extensions affect the edges of sites and (d) poor distribution of agricultural inputs and water affect the site homogeneity. Poor homogeneity in especially soil moisture supply can destroy feild screening. Hence, the experimental field selected for drought phenotyping should be assured for maximum possible homogeneity. The field heterogeneity can be assessed by micro-level soil profiling and mapping.

(ii) *Experimental station fault:* The crop growing conditions are often different from those in the farmer's field. The experimental stations are characterized by high soil fertility, which is not typical of dryland farming. Influence of non-conventional crop grown in the previous season can influence the experiment.

(iii) *Controlling moisture regime:* Control over the moisture or salt regime is achieved by eliminating undesirable rainfall during experimental period using rain-out shelters.

(iv) *Replicating the experiments:* Repeating the screening/evaluation trails in the desired environments and follow suitable analysis methodology will increase the precision of screening.

5.1 Conventional Breeding Approach

The crop resistance to stress may be defined as the mechanism(s) causing minimum yield loss in a stress situation relative to the maximum yield in a stress-free environment. The conventional or empirical breeding approach for abiotic stress tolerance is based on the selection of yield and its components under a given environment (Serraj *et al*., 2003). A commonly followed conventional method of selecting plants for heat-stress tolerance is to grow breeding materials in a hot target production environment and identify individuals/lines with greater yield potential (Ehlers and Hall, 1998). Native varietal halo-tolerance was exploited to characterize differences between salt-sensitive and salt-tolerant barley varieties (Hurkman *et al*., 1989). In India, the salt tolerant lines developed in wheat are *Kharchia* and *Rata*. Salt-tolerant varieties developed in rice by pure line selections from local traditional cultivars are *Damodar* (CSR 1), *Dasal* (CSR 2), *Getu* (CSR 3), *Hamilton* and by pedigree method, namely, CSR 10, CSR13 CSR23, CSR27 and CSR36. In case of rice, the salinity-resistant varieties have been developed by crossing with *Pokkali*, a salinity-resistant local landrace (Sairam and Tyagi, 2004). Through conventional breeding, number of wheat and maize drought tolerant varieties was developed by CIMMYT and adapted in African countries.

Ideotype or trait based approach: The trait based breeding programme can help to improve the crop adaptation and to enhance productivity under abiotic stress conditions. In breeding for stress tolerant types combining empherical selection assisted with physiological traits will be of more advantages (Reynolds *et al*., 2000; Araus *et al*., 2002) in the following respects.

- Helps to eliminate inferior agronomic phenotypes at an early generation itself.
- Selecting superior physiological phenotypes using rapid detection techniques in intermediate generations.
- Selecting for higher performance types in yield trials in advanced generations.

In ideotype approach, plant types preferred for different abiotic situation is decided in advance. Accordingly, the suitable donor source is identified and transferred through elite genetic background. Plant types preferred for different drought stress scenario and timing of stress are extracted from Blum (2011) and the concise details are presented in Table 1.

Table 1 The different plant types preferred for drought tolerance.

Stress situations	*Ideotype preferred*
1. Drought situations	
i. Soil moisture is always available at depth (sub-soil moisture)	Deep rooted genotype[1]
ii. Water is available at depth, but soil is hard and offers large resistant to root penetration	Deep rooted genotypes[2]
iii. Stored soil moisture with limited or no seasonal rainfall	Genotypes with reduced plant size and early flowering
iv. Water is not available in depth	Genotypes with large root length density and small root (hydraulic) resistance[3]
2. Timing of drought stress	
i. Seedling development stage	Selecting better seedling survival types[4]
ii. Vegetative (pre-flowering) growth stage	(a) Genotypes able to continue growing during stress and/or be capable of recover upon rehydration. (b) Genotypes with dehydration avoidance[5]
iii. Flowering stage (most sensitive stage)	Low plant ABA status[6]. Delayed heading or flowering, short anthesis-to-silking interval (ASI) in maize
iv. Fruit or grain development stage	Genotypes with non-senescence (stay-green) or stem reserve utilization for grain filling

[1]In case of cereals, limited tillering at the vegetative stage can enhance deeper root development especially when top soil dries out.

[2]Hard soil resistance can constrain root growth into deep soil despite genotype has ability to grow long roots. Such situations management practice (deep tillage) offer solution to this problem.

[3]For greater soil moisture extraction by roots under lower soil water potential.

[4]By looking into constitutive accumulation of osmotically active solutes and other protective agents in seedlings.

[5]By way of maintaining plant water potential or by osmotic adjustment as reflected by RWC.

[6]Observing phenotypic expression of fertility dysfunction, because the ABA accumulation promotes stomatal closure but it causes distinct sterility symptom during flowering stage stress.

A conceptual model for drought tolerant ideotype in wheat was proposed by Reynolds *et.al*., (2001a) with high expression of the following traits, but not all of which would be useful in all drought environments:

- Seed size and coleoptile length to improve early crop establishment.
- Leaf anatomical traits, e.g., waxiness, pubescence, rolling, thickness (reduce risk of photo-inhibition), high tiller survival and stay-green.
- Early ground cover and pre-anthesis biomass to reduce evaporation of soil moisture.
- Stem reserves/remobilization and spike photosynthesis that help grain filling during severe post-anthesis stress.
- High stomatal conductance as an indicative of roots which are able to extract soil water at depth.
- High osmotic adjustment to maintain cell functions at low water potential.
- Accumulation of abscisic acid for pre-adapts cells to stress.
- Heat tolerance (heat stress may be caused by low leaf transpiration rates under drought).

Difficulties encountered in conventional breeding approach for abiotic stress tolerance are (a) the abiotic stress tolerant traits are mostly governed by polygenes and are difficult to transfer through simple breeding programmes, (b) linkage drag of negative alleles/trait from donor parent to recurrent parent is another major cause of concern, and (c) handling large number of segregating population and identifying the desired genotype by simple empirical selection is difficult.

5.2 Tailoring Abiotic Stress Tolerant Traits by Gene or Traits Pyramiding

In climate changing era, the job of plant breeder is to device the suitable strategy to incorporate the tolerant traits in suitable genetic background in a reasonably shorter time. The choice of trait to be transferred for abiotic tolerance varies with drought or stress situation and time of stress. Both marker-assisted breeding and genetic engineering strategies can be efficiently used for trait pyramiding. Molecular marker technologies can be used to dissect defined quantitative traits by quantitative trait locus (QTL), so that individual loci can be targeted in marker-assisted selection (Witcombe *et al*., 2008).

Marker-assisted trait/QTL pyramiding: It is easy to pyramid genes controlling qualitative traits like disease and pest resistance having major effect on phenotype and easy to measure. Whereas, most of the traits confering abiotic stress tolerance are quantitative in nature. Hence, molecular markers and QTL help in screening and introgression programmes. Large QTLs were identified and reported for the abiotic stress tolerant traits (ASTTs) such as root traits in rice and maize, osmotic adjustment and leaf rolling in rice, leaf ABA concentration and anthesis silking index in maize, stay-green type in sorghum, rice and wheat. The QTLs for submerged tolerance in rice, cold and salt tolerance in rice and wheat were also identified. The list of QTLs identified for abiotic stress tolerant traits and stress responsive genes in different crops is available in biotech issues at ‘www.plantstress.com’. QTL(s) identified for the stay-green character (Kumar *et al.,* 2010), senescence-related traits (Vijayalakshmi *et al.,* 2010) and canopy temperature under heat (Pinto *et al.,* 2010) in wheat.

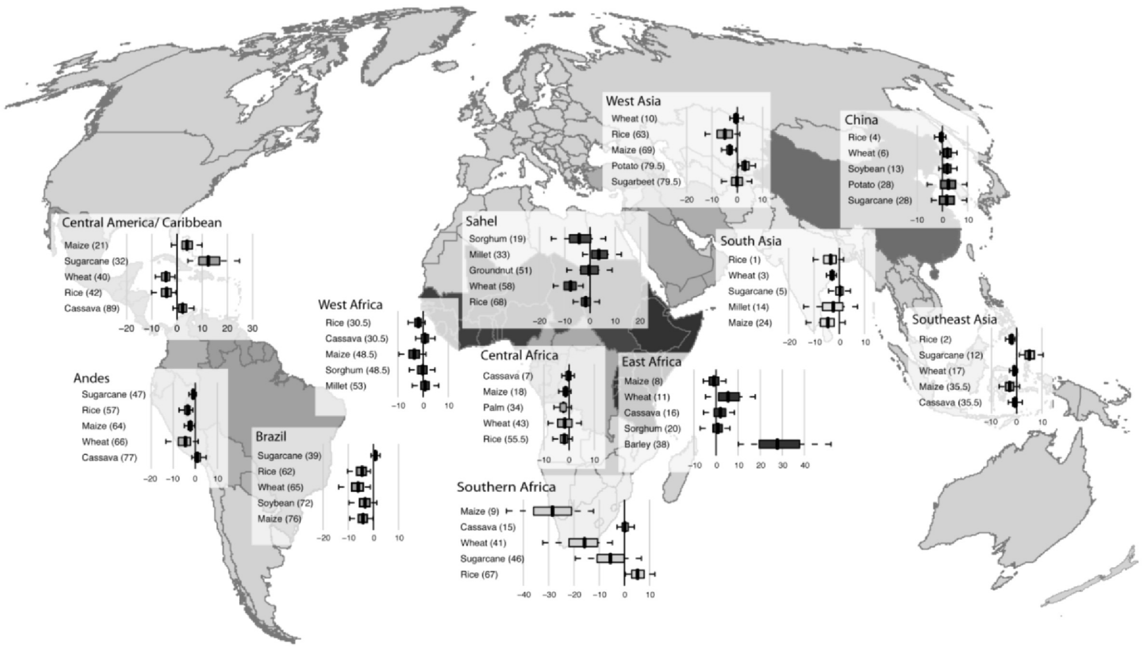

Fig. 1 Projected impacts of climate change by 2030 for five major crops in each region. For each crop, the dark vertical line indicates the middle value out of 100 separate model projections, boxes extend from 25th to 75th percentiles, and horizontal lines extend from 5th to 95th percentiles. Number in parentheses is the overall rank of the crop in terms of importance to food security, calculated by multiplying the number of malnourished in the region by the per cent of calories derived from that crop (adaopted with permission from Lobell *et al.*,)

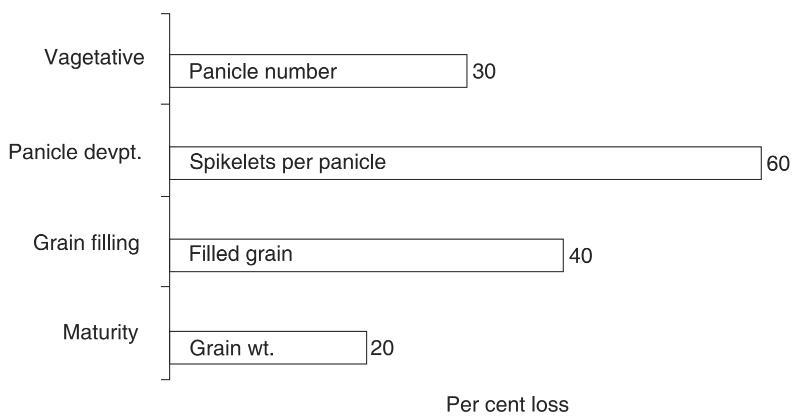

Fig. 2 Impact of water stress at different growth phases in rice

The marker-assisted selection (MAS) comes in handy, if the marker is linked to the target genes. MAS is useful for (a) simply inherited traits, but difficult to phenotype or do not have consistent phenotypic expression under certain specific selection environment; (b) if the major genes affecting the quantitative trait are identified, it is easy to pyramid those genes by marker-assisted breeding; (c) the genes have similar phenotypic effect can be pyramid through MAS, otherwise difficult in conventional phenotypic selection, and MAS helps to control the linkage drag and accelerated the recovery of recurrent genome (Ye and Smith, 2008). Different marker assisted breeding methods are used to accumulate abiotic stress tolerant trait QTLs into the desirable genetic background is describe below:

(i) *Marker-assisted backcross breeding (MABC):* This method is used to transfer desirable ASTT governed by major genes/QTLs from donor to agronomically superior recurrent parent. It needs genetic markers that are associated with genes or QTLs affecting whole plant stress tolerance or individual components contributing to it. The steps involved (Kulwal *et al*., 2011) in MABC are: (a) identification of molecular markers associated with ASTTs, (b) validation of identified markers in the genetic background of targeted genotype (recurrent parent) to be improved, and (c) MABC to transfer the QTL/gene from donor into the recurrent parent.

MABC combines 'foreground' selection of donor alleles linked to QTLs and 'background' selection of recurrent parent alleles in the BC_2 and later generations. Foreground selection involves the use of two flanking markers for selecting a particular gene or a QTL. Background selection uses markers that are not associated with the desirable QTL/gene and selects backcross progeny with the highest proportion of genome of the recurrent parent (Witcombe *et al*., 2008; Gaur *et al*., 2012). The stepwise and simultaneous-stepwise transfer of abiotic stress tolerant trait(s) to recurrent parent is schematically represented in Figs. 4 and 5, respectively. The improved cultivars of submerged tolerance was developed using *Sub 1* gene/QTL in rice (Septiningsih *et al*., 2009) and for pre-harvest sprouting tolerance in wheat (Kumar *et al*., 2010a) by marker-assisted introgression.

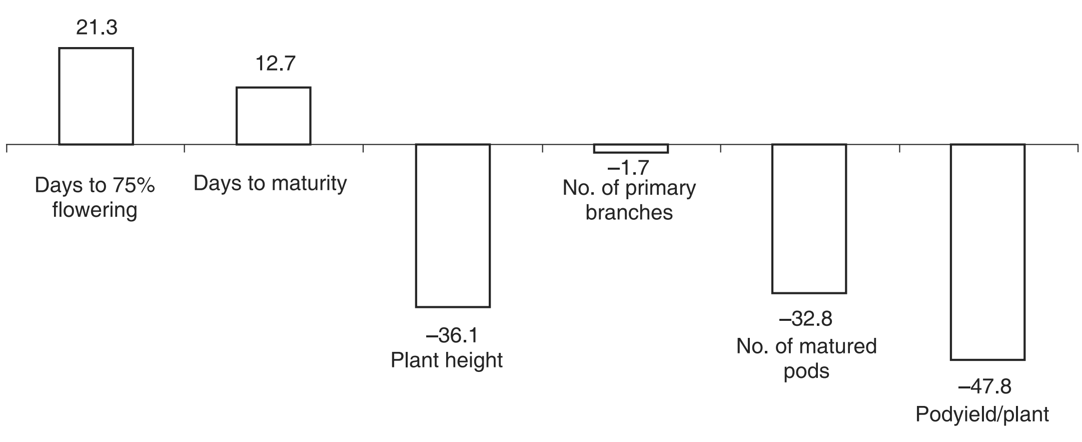

Fig. 3 Per cent influence of soil moisture stress during flowering and pod development stages in peanut traits

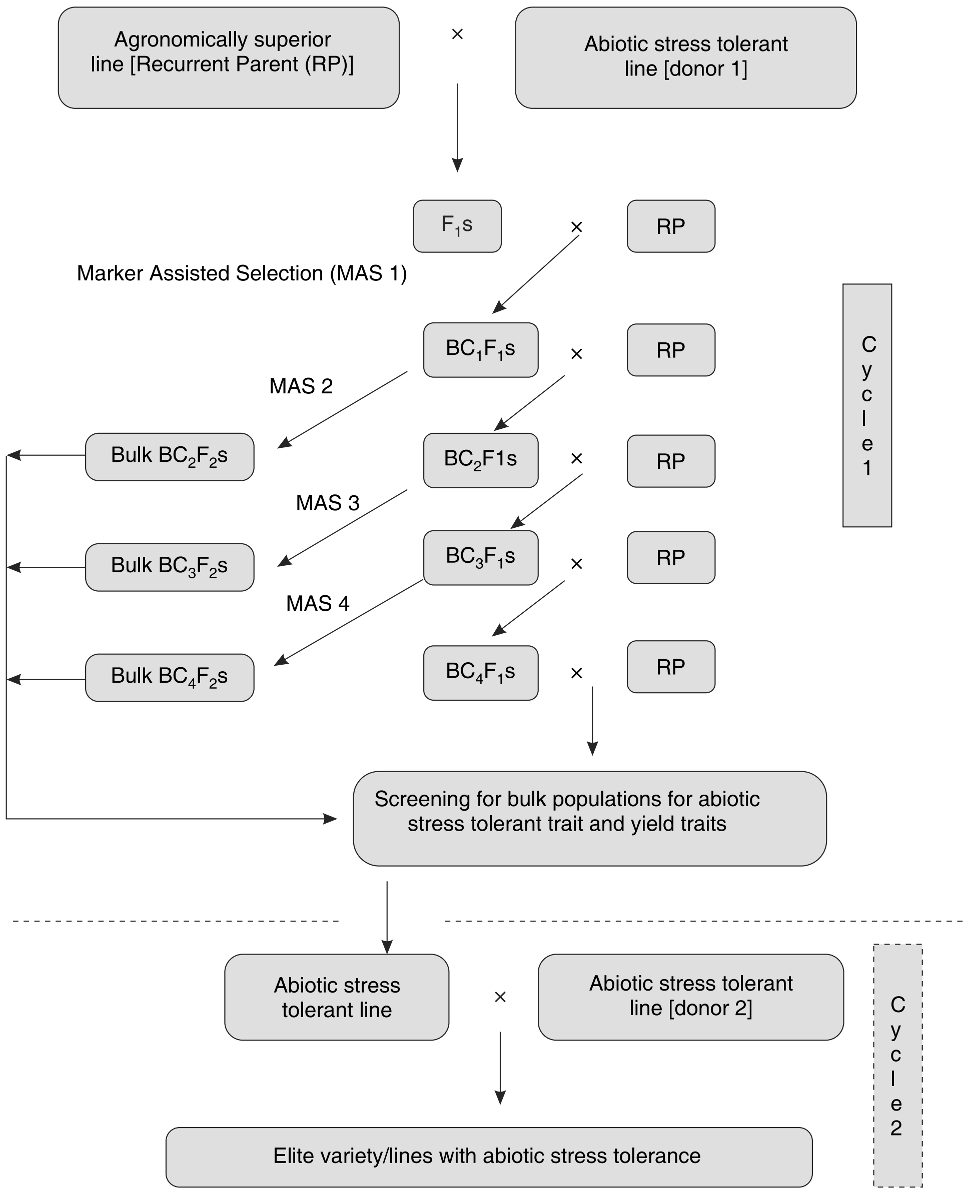

Fig. 4 Stepwise transfer of abiotic stress tolerant trait(s) by marker assisted backcross

(ii) *Marker-assisted gene pyramiding by multiple cross:* In this method, genes of desirable trait from multiple parents are converged into a single genotype to enhance desirable trait performance. Evolving of abiotic tolerant lines through this method in a single breeding cycle involves creation of root genotype by two-or three-way crosses. This brings desirable genes into single genotype as heterozygous from different parents. Then fix these genes

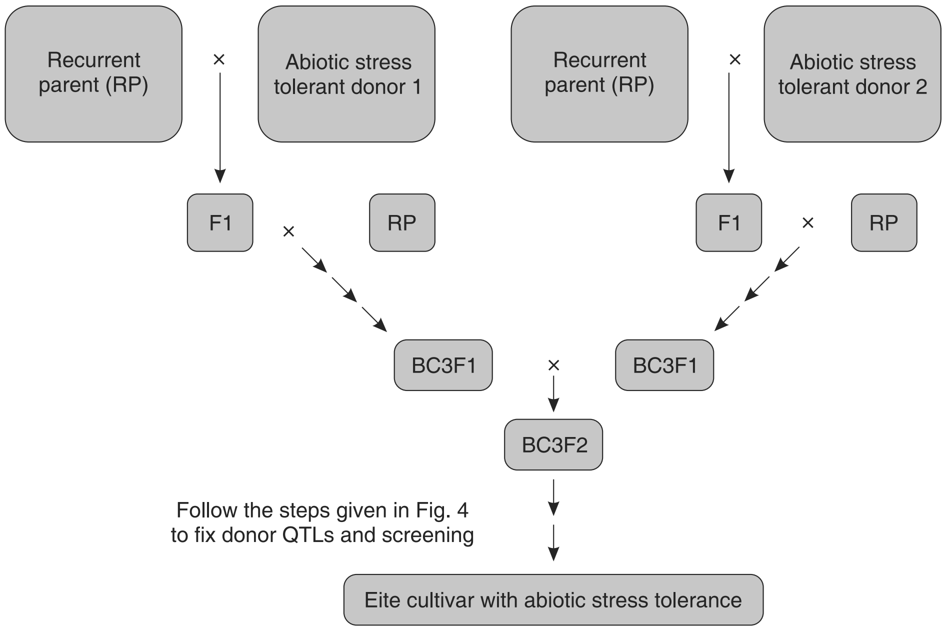

Fig. 5 Simultaneous and stepwise transfer of abiotic stress tolerant trait(s) by marker assisted selection

as homozygous state in inbred lines by selfing or double haploid strategies. To ensure the presence of genes of target traits, it is essential to genotype by MAS in all the steps of breeding cycle. Bringing four favourable genes of an abiotic stress tolerant trait from different parents to develop elite inbred line(s) is represented in Fig. 6.

(iii) *Marker-assisted recurrent selection (MARS):* Most of the ASTTs are quantitative in nature, and are governed by polygenes with minor effects. The transfer of such minor genes/ QTLs through MABC is much difficult as a large number of progenies are needed to select desirable ones. In such situation, the MARS will be of very useful in pyramiding desirable alleles in single genotype. Conventionally the recurrent selection is used to accumulate the desirable alleles for yield and other ploygenic traits in cross-pollinated crops. In MARS, the individuals for intercrossing are selected using selection index-constructed, based on QTL associated markers. The genetic gain using such selection index is higher than the conventional phenotypic selection (Gaur *et al*., 2012).

(iv) *Advanced backcross QTL analysis (AB-QTL):* This method was proposed by Tankley and Nelson (1996), and is useful for simultaneously to identify and introgress favourable alleles from unexplored donors into elite genetic background. This method readily applied to annual crops and is difficult to apply to crops for which inbred lines do not exist. It would be difficult to apply this method to highly heterozygous, cross-pollinated and clonally propagated crops. The advantages of AB-QTL analysis for introgression of valuable QTLs as compared with conventional strategies for QTL detection is discussed by Tanksley and Nelson (1996). If the identified markers are available for abiotic stress tolerant traits,

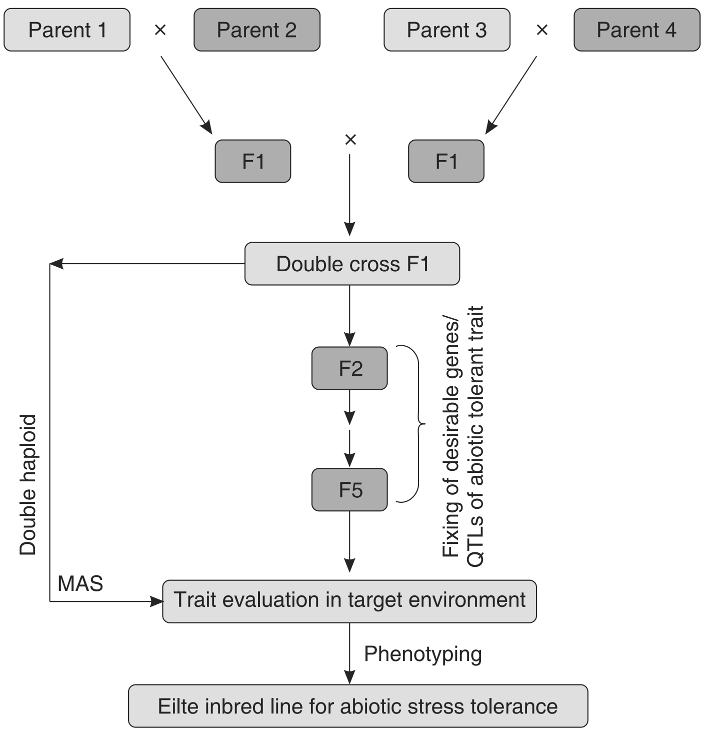

Fig. 6 Pyramiding of four genes/QTLs of a quantitative trait for abiotic stress by double cross and marker-assisted selection

the AB-QTL strategy can be utilized to transfer such traits into agronomically superior varieties. The steps involved in this method are narrated below:

- The commercially preferred cultivar/recurrent parent is crossed with wild species or donor parent.
- Backcrossing is effected with recurrent parent up to BC_2 or BC_3 generations. This method adopts strategy of delay the QTL and marker analysis to BC_2 or BC_3, generations beyond the BC_3 are likely to have low statistical power to detect most QTLs. The molecular marker/QTLs are used to conduct negative genotype and phenotype to eliminate the undesirable alleles from donor parent (Grandillo and Tanksley, 2003). This allows to reduce the frequency of the donor-parent genome in each of the advanced backcross lines and to avoid the masking effect from deleterious wild recessive alleles or donor parent.
- The BC_2 or BC_3 are backcrossed with donor parent to generate BC_2F_1 or BC_3F_1 population in hybrid crops (BC_2S_1 or BC_3S_1 for open-pollinated crops).
- Evaluation of BC_2F_1 or BC_3F_1 families to be carried out for stress tolerant and yield traits under monitoring of QTLs. Once favourable QTLs are identified not more than two generations required to isolate targeted QTL-NILs.
- Evaluation of NILs is done for stress tolerant and yield traits under target environment to identify the elite lines and for varietal development.

(v) *Genomic selection (GS):* The advantage of genome-wide or genomic selection is that it does not need to workout the marker-trait association as required in MABC or MARS. It calculates the marker effects (genomic estimated breeding values) of lines across the entire genome which explains the entire phenotypic variations of particular trait (Kulwal *et al*., 2011). The superior lines can be selected based on breeding values to advance the progenies. Mayor and Bernardo (2009) have shown that double haploid populations are very useful in genome-wide selection compared to F_2 populations, especially for complex traits that are controlled by many QTL. The in-built advantages of this method can be effectively exploited for the abiotic stress breeding to evolve tolerant cultivars in a quicker time.

(vi) *MAPS breeding scheme:* Zong and coworkers proposed a novel QTL pyramid breeding scheme based on marker-assisted and phenotype selection (MAPS) that allowed pyramiding of as many as 24 QTLs at a single hybridization without massive crossing work. The MAPS QTL pyramiding scheme, not only overcomes the shortcomings of crossing NILs, but also efficiently pyramid the desired QTLs without any backcrossing. Combining the genome-wide association studies (GWAS) results with MAPS QTL pyramiding scheme, it is possible to breed stress tolerant lines in a novel way by assembling more number of QTLs as detailed by Zong *et al*., (2012) in rice.

5.3 Trait Pyramiding by Transgenic Approach

The recent advances in plant molecular biology have thrown out a number of genes associated with abiotic stress mechanisms/adaptive traits. Some of these genes were validated for their function. In case of drought stress, the tolerance mechanism is amenable to genetic engineering of specific genes. However, whole-plant avoidance strategies depend more on conventional breeding schemes and QTL analysis (Vinocur and Altman, 2005). The major requirement for developing drought/abiotic stress resistance cultivar through transgenic approach are (a) the genes identified should improve drought resistance significantly; (b) no phenotypic changes for other traits; (c) no yield penalty under non-stress conditions; and (d) ensure over-expression of a single copy transgene rather than multiple copies of a gene that can leads to instability of expression and inheritance or even gene silencing (Xiao *et al*., 2007; Blum, 2011). The transgenic lines were produced for over-expression of ion transport proteins; osmoprotectants mechanism for improving salt tolerance is reviewed in detail by Ashraf and Akram (2009). The stress adaptive genes can be exploited through genetic engineering of 'designer' traits as narrated below:

(i) *Use of abiotic stress tolerant genotypes for transgenics:* Identify the genotype(s) with superior water relation (drought avoidance) and use them as recipient genotype to develop transgenic. Transfer and express validated genes regulating other abiotic stress adaptive mechanism (cellular tolerance) in recipient background (Karaba *et al*., 2011), and follow stringent evaluation for stress tolerance to identify elite genotypes (Fig. 7).

(ii) *Co-expression of validated abiotic stress genes:* Through currently available transformation technology, it is possible to transfer multiple genes, which may act synergistically and additively to improve plant stress tolerance (Wahid *et al*., 2007; Karaba *et al*., 2011). Co-express validated genes regulating diverse mechanisms using multi-gene expression cassettes. Co-expression of multi-genes into plant genome can be achieved by the various

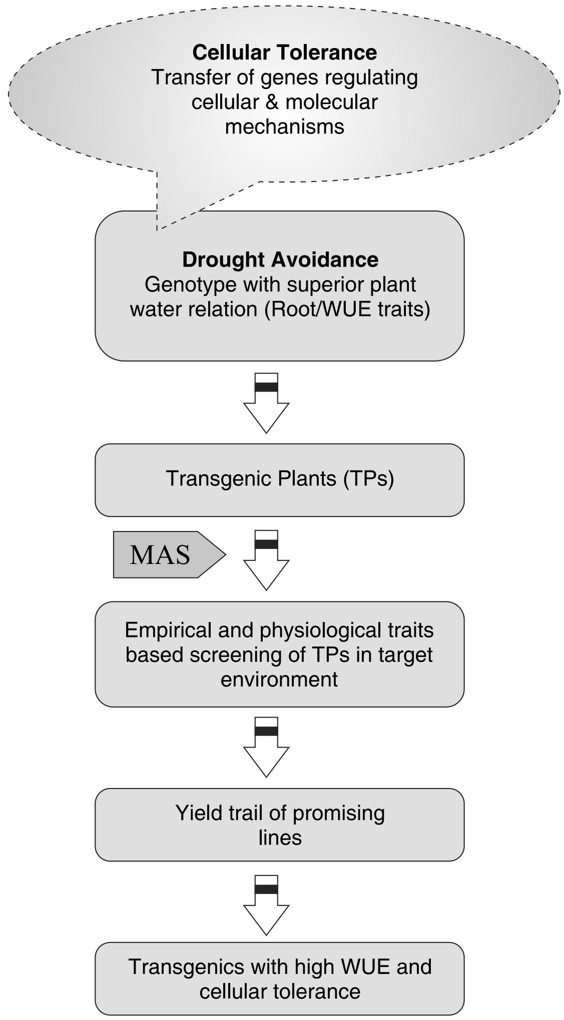

Fig. 7 Pyramiding of drought tolerant trait(s) by transgenic approach

strategies such as (a) crossing between two transgenic plants; (b) co-transformation with multiple genes; (c) transformation of single plasmid carrying several linked transgenes or multi-genes; and (d) polycistron or polyprotein strategy.

6. IMPACT OF CLIMATE CHANGE ON PLANT GENETIC DIVERSITY

Genetic diversity in crop plants leads agriculture to withstand moderate climate changes experienced over past year (Lane and Jarvis, 2007). The erosion of crop biodiversity (so called genetic erosion) by the human intervention and modern agricultural practices threatened the changes for naturally adapted varieties/land races. To exploit the wealth of gene sequence information provided by the 'genomics revolution' in major crops and mine agricultural germplasm for genetic diversity, high resolution, high throughput technologies in plant physiology are required for bridging the gap between genotype and phenotype (Furbank, 2009).

Crop production systems that rely on highly selected genetic resources might be increasingly vulnerable to climate change impacts such as epidemic disease spread. If the production trend declines, there will be pressure on marginal lands to cultivate or implement unsustainable practices that over the long term, degrade lands and resources, and adversely impact biodiversity on and near agricultural areas. In this regard, already food insecure people in the developing countries will be most adversely affected by climate change. These changes have been seen to cause a decrease in the variability of those loci controlling physical responses to climate (Jump *et al*., 2005). Therefore genetic variation holds the key to the ability of populations and species to persist over evolutionary time through changing environments (Shaffer *et al*., 1985). No organism can predict the future (and evolutionary theory does not require them to), nor can any organism be optimally adapted for all environmental conditions. Nonetheless, the current genetic composition of a species influences how well its members will adapt to future physical and biotic environments.

The population can "migrate" across the landscape over generations. By contrast, populations that have a narrower range of genotypes (and are more phenotypically uniform) may simply fail to survive and reproduce at all as conditions become less locally favourable. Such populations are more likely to become extirpated (locally extinct), and in extreme cases the entire species may end up at risk of extinction. For example, the Florida Yew (*Torreya taxifolia*) is currently one of the rarest conifer species in North America. But in the early Holocene (10,000 years ago), when conditions in southeastern North America were cooler and wetter than today, the species was probably widespread. For reasons that are not completely understood, *T. taxifolia* failed to migrate northward as climate changed during the Holocene. Today, it is restricted to a few locations in the Apalachicola River Basin in southern Georgia and the Florida panhandle. As the *T. taxifolia* story illustrates, once species are pushed into marginal habitat at the limitations of their physiological tolerance, they may enter an "extinction vortex," a downward cycle of small populations, and so on (Shaffer *et al*., 1985; Gilpin *et al*., 1986). Reduced genetic variability is a key step in the extinction vortex. Gene-banks must be better to respond to novel and increased demands on germplasm for adapting agriculture to climate change. Researchers informed that 16-22% of wild species might go extinct (Jarvis *et al*., 2005). Some species can adapt to projected future environments, some cannot this referred to as phenotypic plasticity. Therefore monitoring changes in crop (any) biodiversity is desirable. It is urged to have collaborative effort of public and private sector researchers to broaden and enhance the cultivated crop germplasm base. Gene-banks need to include different characteristics in their screening processes and their collections need to be comprehensive, including minor crops. Thus breeding efforts will require the continued collection, evaluation, deployment and conservation of diverse crop genetic material to sustain the crop production.

7. CONCLUSION

Abiotic stress breeding demands an integrated effort of plant breeder, crop physiologist, molecular biologist and soil scientist to evolve climate flexible cultivars. The success of abiotic stress breeding is lies in (a) adapting suitable methodologies to impose the stress at required intensity, desired stage of crop growth and duration; (b) choosing appropriate screening tools to assess the response of abiotic tolerant trait/mechanism under the given environment; (c) identifying genotypes with abiotic stress tolerant traits suited for stress scenario as a donor by stringent screening; (d) converge different traits that confers abiotic stress tolerance in a agronomically superior genetic background through modern crop improvement tools for rapid advancement; and (e) validating the elite genotypes with 'designer' traits under target stress environments before releasing as a variety. The available high-throughput techniques can be effectively used for genotyping and phenotyping to screening large number of genotypes and to identify small differences in targeted traits. It is better to select parents for breeding programme, only after the results of controlled condition are validated in field conditions.

The breeders' business does not rest with converging different abiotic stress tolerant traits in evolving climate-resilient varieties to mitigate the climate change, but also ensure the produce quality requirement of different communities. The crop breeding only can offer limited solution to climate change in short term; hence further emphasis should be given for the climate-smart agricultural practices such as conservation tillage, residue management, building soil organic matter, agro-forestry, efficient use of water can further help to manage stress like drought and salinity. Identifying and executing cost-effective and rapid genotyping and phenotyping methods in near future will help to evolve climate adapted cultivars, which hopefully address the climate change challenges in agriculture in short term. Plant biodiversity is yet another reservoir of variable adaptive traits for long-term solution and need to be conserved with well-database management in every locality to save from natural disasters including climate change.

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