Review

Breeding for abiotic stress tolerance in chrysanthemum (*Dendranthema* x *grandiflora* Tzvelev)

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ABSTRACT

Chrysanthemum (*Chrysanthemum* x *grandiflorum* Tzvelev) is one of the most important ornamental flowers and occupies an important position as cut flowers, potted and ground-cover plants in the world. In India it is also used for making garlands, Venis, Gajaras and for religious offerings. The productivity and quality of plants are mainly influenced by environmental stress especially by heat, cold, drought and salt stress. The improvement of its tolerance to these abiotic stresses is a priority for breeders and thus various approaches have been used to develop abiotic stress tolerance in chrysanthemum. Wide distant (interspecific and intergeneric) hybridization has contributed positively to improve drought, salt and heat tolerance in chrysanthemum through introducing abiotic stress-tolerant trait from its wild relatives. In vitro mutagenesis is another important technique that combines both tissue culture technique and mutation for inducing stress tolerance and thus improves the yield and quality of the plant. The use of genetic engineering technology for creating abiotic stress tolerant chrysanthemum by the introduction of novel trait has proven to be the potential approach when it is difficult to achieve through a conventional breeding programme.

Keywords: Abiotic stress; chrysanthemum; genetic engineering; hybridization; mutagenesis

INTRODUCTION

Abiotic stress management is one in every of the foremost vital challenges faced in agriculture. Various abiotic factors like low water availability (drought), excess water (flooding/water logging), extremes of temperatures (cold, chilling, frost and heat), salinity, mineral deficiency and toxicity challenge the crop production. The negative effects of abiotic stresses bring about changes in plant metabolism, growth and development and in extreme cases leads to plant death (Khan et al 2014). This has become a specific concern in chrysanthemum as well where stress-related changes limit productivity and can lead to intolerable economic loss.

Chrysanthemum (*Dendranthema* x *grandiflora* Tzvelev) belonging to family Asteraceae is an important ornamental plant in the global floriculture industry both as a cut flower and as a pot plant. It ranks second in the international cut flower trade (Datta and Gupta 2012) after rose. Due to its wide range of colour, form and excellent keeping quality, chrysanthemum has earned incredible popularity in floriculture industry. It is believed to be a native of the Arctic parts of north and central Russia, Japan and China. The genus consists of more than 40 species distributed mainly in east Asia and occupies an important position as cut flowers, potted and ground-cover plants in the world. In addition it is highly demanded for making garlands, Venis, Gajaras and various religious offerings in India. But the supply of flower is limited by the intolerance of chrysanthemum to abiotic stresses viz heat, cold, drought and salt stress (Li 1993). The tolerance of plants to withstand abiotic stress varies with physiological growth stage, biological developmental stage and size of plants. Low temperature, drought and salt stress limit planting areas of chrysanthemum (Hong et al 2006a). Hence it is very necessary to improve heat, cold, drought and salt tolerance in chrysanthemum.

The characteristics of abiotic stress may vary considerably depending on the location and degree of stress and also likely to vary during the crop season. A given abiotic factor may increase or decrease the level of another abiotic stress for example in saline soil, moisture stress would enhance salinity stress (Dhakar et al 2017). The different species of chrysanthemum show difference in their ability to tolerate the abiotic stresses. For instance *Chrysanthemum grandiflorum* Yuhuaxingchen is an important commercial chrysanthemum cultivar with an excellent ornamental quality but has low drought tolerance whereas *C indicum* has exceptional drought tolerance (Sun et al 2010a). Sometimes effects generated by one abiotic stress may overlap with some effects of another stress.

Major abiotic stresses expressed by plants

Drought

In a natural environment, drought and heat stresses occur in concordance and often hamper the phenotyping processes in the crop improvement programme. The scarcity of water often hinders the plant productivity. Few plants have the ability to adapt under sub-optimal water supply without hindering the vegetative and reproductive stages without affecting the economic yield; such plants are considered to be drought tolerant. Various factors govern drought stresses like extremes in temperature, photon irradiance and paucity of water or low water potential due to high solute concentration. The results are: reduced leaf size, stems extension and root proliferation which in turn disturb plant water relations and ultimately reduce the water-use efficiency. When drought stress prevails, plants express complex phenomenon displaying a variety of physiological and biochemical responses at cellular and whole-organism levels. The activity of various enzymes gets hampered particularly CO_2 fixation, adenosine triphosphate synthesis and other physiological factors such as stomatal closure, membrane damage and CO_2 assimilation by leaves are reduced (Farooq et al 2009). Tolerant plants initiate defence mechanisms against water deficit in order to cope up with the drought (Chaves and Oliveira 2004). Henceforth it is crucial to improve the drought tolerance of crops under the changing environment. Till date there are no economically viable technological means to facilitate crop production under drought. However the development of plant varieties tolerant to drought stress might be a promising approach which helps in meeting the continuous flower demand in the market.

In the world scenario more than 30,000 chrysanthemum cultivars exist however most of them have low drought-tolerance and thus restrict the cultivation of chrysanthemum under water-deficit areas as it reduces the flower quality and increases the cost of production (Chen et al 1995, Zhang et al 2005). These problems can be addressed by developing drought tolerant chrysanthemum varieties. This is an economical approach to solve these problems and to reduce the use of freshwater resources for its production. The strategies include mass screening and breeding, marker-assisted selection as well as genetic engineering for drought resistance. Various morphological mechanisms operative under drought conditions are drought escape, drought avoidance and drought tolerance.

Drought escape: With the shortened life cycle or growing season plants can escape drought as it allows plants to reproduce before the environment becomes dry. One of the important traits related to drought adaptation is a flowering time where a short life cycle can lead to drought escape (Araus et al 2002).

Drought avoidance: The drought avoidance mechanism can be either constitutive or induced. The characters such as root biomass, length, density and depth are the main drought avoidance traits to avoid drought by the plants (Subbarao et al 1995, Turner et al 2001). In addition waxiness on leaves is considered as a desirable trait for drought tolerance as it helps in the maintenance of high tissue water potential (Richards et al 1986, Ludlow and Muchow 1990). According to report of Sun et al (2010a) the density of the leaf epidermal hair may be useful selection criterian for drought-tolerant evaluation in chrysanthemum breeding programmes. In addition the extensive and prolific root system that maintains the water uptake and stomatal control of transpiration are the other mechanisms that reduce water loss from plants (Turner et al 2001, Kavar et al 2007).

Drought tolerance: Physiological mechanisms like osmotic adjustment, osmo-protection, antioxidation and a scavenging defence system have been the most important bases responsible for drought tolerance. Drought tolerant plant has the ability to withstand water-deficit with low tissue water potential. The plant achieves drought tolerance through maintenance of turgor by osmotic adjustment, increase in elasticity in the cell and decrease in cell size. Kavar et al (2007) reported that a stress condition directly or indirectly triggered the secondary stresses and/or injury responses which then lead to changes in gene expression (up and down-regulation) at a molecular level. These gene products thus obtained are thought to function as tolerance to drought.

Heat stress

The effects of heat stress vary considerably at different developmental stages of plant and with the intensity of the stress like during seed germination the high temperature may slow down or totally inhibit germination. However at the later developmental stages it may adversely affect photosynthesis, respiration, water relations and membrane stability and also modulate levels of hormones and primary and secondary metabolites. The rise in temperature beyond a threshold level for a period of time sufficient to cause irreversible damage to plant growth and development is often defined as heat stress. Wahid et al (2007) reported that a transient elevation in temperature usually 10-15°C above ambient is considered heat shock or heat stress.

In order to cope up with heat stress, the plants implement various mechanisms like maintenance of membrane stability, scavenging of reactive oxygen species (ROS), production of antioxidants, accumulation and adjustment of compatible solutes, induction of mitogen-activated protein kinase (MAPK) and calcium-dependent protein kinase (CDPK) cascades and most importantly chaperone signalling and transcriptional activation. The adverse effect of heat stress can be mitigated by adopting various breeding approaches for developing crop plants with improved thermo-tolerance.

In the scenario of chrysanthemum Wang et al (2008) observed the reduction or delay in flowering under high temperatures in many cultivars. Heat stress also inhibits chrysanthemum growth, flower bud differentiation and pollen development and causes delayed inflorescence and abnormal flowers (Cockshull and Kofranek 1994, Adachi et al 2000). For commercial production, the genotypes bearing heat-tolerant and flowering-heat-delay insensitive need to be identified and selected. The grower in the subtropical areas divides chrysanthemum as heat-tolerant (flowering during the natural summer and fall seasons) and heat-intolerant (flowering during the natural winter and spring seasons) for year-round production. Therefore breeding heat-tolerant or heat-delay-insensitive genotypes is vital because the summer temperature is high in all growing conditions either in greenhouses or in the field. Anderson and Ascher (2001) suggested that heat-delay-insensitive chrysanthemum genotypes however rapid and effective, seedling screening either in greenhouse or field evaluation techniques is rampant. However Shibata and Kawata (1987) crossed summer-flowering chrysanthemums and year-round types and obtained seedling clones that normally and regularly flowered under high-temperature conditions.

Cold stress

In chrysanthemum cold tolerance trait is becoming increasingly desirable with the current rise in demand for winter-hardy herbaceous perennials but many chrysanthemum species or varieties lackscold-tolerance (Anderson and Gesick 2004). Generally cold stress in plants may lead to poor germination, stunted seedlings, yellowing of leaves, reduced leaf expansion and wilting and even the death of tissues (necrosis). Low temperature particularly damages the roots and rhizomes of field grown plants (Anderson and Gesick 2004). Widmer (1958) found that exposure to -10 to -15°C caused injury to acclimated rhizomes of winter hardy cultivars. The plant maintains homeostasis to acquire freezing the tolerance which involves extensive reprogramming of gene expression and metabolism in order to cope up with cold stress. The chrysanthemum lacks cold tolerance within the primary gene pool henceforth a combination of mutagenesis, transgenics and wide hybridization appears to be required to address this failing.

Salt stress

Salinity stress limits crop yield affecting plant growth and restricting the use of land. Soil salinity affects the plant in two major ways: firstly a high concentration of salts in the soil which makes it harder for plant roots to extract water and second high concentration of salts within the plant can be toxic to the plants. Plant growth responds to salinity in osmotic phase that inhibits growth of young leaves and in an ionic phase that accelerates senescence of mature leaves. The plant adapts to salinity by osmotic stress tolerance, Na^+ or Cl^- exclusion and the tolerance of tissue to accumulated Na^+ or Cl^- (Munns and Tester 2008).

Many chrysanthemum cultivars are available worldwide but most of them unfortunately are susceptible to abiotic stress including salt stress causing serious crop losses and hence reduction of the yields by more than half (Bray et al 2000). The salinity tolerance breeding has been attempted by Flowers

(2004) through conventional breeding programmes with the use of in vitro selection, interspecific hybridization, through marker aided selection as well as by the use of transgenic plants.

Breeding methods for abiotic stress tolerance in chrysanthemum

Hybridization

Plant hybridization is the process of crossing between genetically dissimilar parents to produce a hybrid. The cross may be between individuals of different species (interspecific hybridization) or genetically divergent individuals from the same species (intraspecific hybridization). Offspring produced by hybridization may be fertile, partially fertile or sterile.

Interspecific hybridization: Interspecific hybridization offers opportunities to transfer useful genetic variation to elite germplasm. It has become a promising strategy to improve drought tolerance of some crops through introducing drought-tolerant trait from their wild relatives into them (Cattivelli et al 2008). In study on interspecific hybridization between *Chrysanthemum grandiflorum* and *C indicum* by Sun et al (2010a) the evaluation for drought tolerance among the hybrids was found to be promising. The reproductive barriers often exist in crosses between chrysanthemum cultivars and their wild species and seriously influence the utilization of excellent genes from the wild germplasm (Sun et al 2010b). The ability to overcome sexual incompatibility has been improved by the elaboration of in vitro ovary and embryo culture (Watanabe 1977) and these techniques have been applied to obtain a number of wide hybrids involving various Asteraceae species (Abd El-Twab and Kondo 2001, Tang et al 2009). To create hybrid tolerant to cold stress an interspecific hybridization between *Dendranthema morifolium* and its wild diploid relative *D nankingense* was done through ovary rescue technique by Cheng et al (2010). The study showed that the cold tolerance of the hybrids was found significantly superior to the parent *D morifolium* suggesting that this technique can be used as an effective means for cultivar improvement in chrysanthemum.

Intergeneric hybridization: The distant hybridisation was successfully carried out with embryo culture between *C morifolium* cultivar Zhongshanjingui (as female) and *Ajania przewalskii* (as male). Through a combination of various assays it was confirmed that the hybrids had cold tolerance which was equivalent to that of the highly tolerant *A przewalskii* parent (Deng et al 2011). Similarly an intergeneric hybridization between *C morifolium* and *Artemisia japonica* was carried out by Zhu et al (2013) via embryo rescue technique from which salt tolerant hybrid was obtained.

In vitro mutagenesis

Plant tissue culture offers natural variations (somaclonal variations) which could further be enhanced by applying various physical and chemical mutagen treatments. An in vitro mutagenesis combines both tissue culture technique and mutation strategy. Through in vitro mutagenesis Hossain et al (2006) developed a table form of NaCl tolerant chrysanthemum (*C morifolium*) using ethylmethanesulfonate (EMS) as a chemical mutagen. In addition the induction of polyploidy has reported as an efficient way to improve abiotic stress tolerance in plants and few studies were reported on improving abiotic stress tolerance in chrysanthemum as well. A tetraploid *Dendranthema nankingense* was induced by the colchicine treatment using nodal segments and found to have enhanced drought and salt tolerance by alleviating oxidative stress, maintained good water balance and higher chlorophyll (a + b) content (Liu et al 2011).

Genetic engineering

For genetic engineering the identification of key genetic determinants having a stress tolerance and introducing these genes into the desired crop are the important steps. Until now many salt-responsive genes have been identified (An et al 2014, Chen et al 2011, Chen et al 2012, Li et al 2015) in various crops. The important transcription factors in plant stress response and signal transduction are dehydration responsive element binding (DREB) proteins. The molecular cloning, expression profiling and trans-activation property studies of a *DREB2*-like gene from chrysanthemum (*Dendranthema vestitum*) indicated that the *DvDREB2A* gene is a new member of the *DREB* transcription factors which may play a crucial role in the development of plant tolerant to environmental stress (Liu et al 2008). Moreover it was found that the over-expression of *Chrysanthemum dichrum CdICE1* gene in *C grandiflorum* conferred the stress tolerance via regulation of *CgDREB* involved in the oxidative and osmotic homeostasis pathways (Chen et al 2012). Similarly increased salinity tolerant chrysanthemum compared with wild type was found when *DgNAC1* transcription factor gene from *C grandiflorum* was over-expressed. It showed better osmotic adjustment, a more effective ROS-scavenging

Table 1. Hybridization programme in chrysanthemum for abiotic stress tolerance

Female parent	Male parent	Characteristic feature	Reference
Interspecific hybridisation			
Chrysanthemum grandiflorum	*Chrysanthemum indicum*	Highly drought tolerant	Sun et al (2010a)
Dendranthema morifolium	*Dendranthema nankingense*	Highly cold tolerant	Cheng et al (2010)
Intergeneric hybridisation			
Chrysanthemum morifolium	*Ajania przewalskii*	Highly tolerant to drought	Deng et al (2011)
Chrysanthemum morifolium	*Artemisia japonica*	Highly tolerant to salt	Zhu et al (2013)

Table 2. Genetic engineering in chrysanthemum for abiotic stress tolerance

Stress	Gene	Origin	Transgenic plant	References
Heat	*AtDREB1A*	Arabidopsis	Chrysanthemum	Hong et al (2009)
Salt, cold and drought	ICE1	*Chrysanthemum dichrum*	*Chrysanthemum grandiflorum*	Chen et al (2012)
Salt, drought and temperature	P5CS1 and P5CS2	*Chrysanthemum lavandulifolium*	*Chrysanthemum × morifolium*	Zhang et al (2014)

system and better protection of the membrane (Wang et al 2017). Therefore *DgNAC1* can be considered as an excellent genetic resource for improving salinity tolerance of plant as it is a positive regulator of salinity tolerance. While He et al (2018) suggested that *DgWRKY2* gene from *Dendranthema grandiflorum* could be used as a reserve gene for salt-tolerant plant breeding as transgenic chrysanthemum showed enhanced antioxidant and osmotic adjustment. Through the transgenic approach Hong et al (2006b) demonstrated that plants transformed with the *Arabidopsis thaliana* gene *AtDREB1A* improved the level of tolerance to low temperature in chrysanthemum in the field condition.

CONCLUSION

The major abiotic stresses like drought, high salinity, cold and heat negatively influence the growth and productivity of a crop. Due to constant climate change it is very difficult to find stress-free areas where crops may advance towards their potential yields. It is desirable that the breeding programme should give high priority for abiotic stress in mainstream breeding programme so that breeding approaches that effectively target stress environments can be gained.

Through ages chrysanthemum has been associated with various world cultures. Considering its importance in our day-to-day life breeders have created numerous genotypes that have made chrysanthemum as top ten cut, potted flowering and garden crops worldwide.

With the constant change in the environmental conditions, breeding for abiotic stress has become a specific concern in chrysanthemum where abiotic stress has limited productivity leading to intolerable economic losses. In chrysanthemum there is a great potential of breeding for abiotic stress resistance through the contributions of wild relatives. Breeding methods like interspecific and intergeneric hybridization, in vitro mutagenesis and genetic engineering for improving abiotic stress tolerance in chrysanthemum have proven to be the potential approaches. This review summarizes the work done on breeding for abiotic stress tolerance in chrysanthemum. This insight is particularly relevant for breeders in low-income countries where due to low socio-economic reasons breeders may not be able to use high-throughput methods in abiotic stress tolerance breeding programmes in chrysanthemum.

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