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Improvement of Horticultural Crops against Abiotic Stresses: A Strategic Approach

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Abstract: ***Abiotic stress factors impose a major threat to agriculture. Therefore, the efforts to develop stress tolerant plants are immense of importance to increase crop productivity. The classical breeding programme are being used to integrate genes into the crop to induce stress tolerance. However, in many cases, it has failed to provide desirable results. In recent years, tissue culture based in vitro selection has emerged as a feasible and cost effective tool for developing stress tolerant plants. Plants tolerant to abiotic stress can be acquired by applying the selection agents such as NaCl (for salt tolerance) and PEG or mannitol (for drought tolerance) in the culture media. Only the explants capable of sustaining such environments survive in the long run are selected. In vitro selection is based on the induction of genetic variation among cells, tissues and organs in cultured and regenerated plants. The selection of somaclonal variations appearing in the regenerated plants may be genetically stable and useful in crop improvement. In this paper, the progress made towards the development of abiotic stress tolerant plants through tissue culture based approaches is reviewed.***

Keywords: Abiotic stress, Somaclonal variation, *in vitro* selection, induced mutation

1. Introduction

Environmental stresses such as high and low temperature, drought, salinity, water deficiency and high water level affect plant growth and decrease horticultural crops productivity worldwide. Damage caused by these stresses are responsible for enormous economic losses worldwide. The warmer climate threatens the production of many horticultural crops, especially those cool-season species. Another major environmental factor that limits crop productivity, mainly in arid and semi-arid region is high salinity. Approximately 20% of irrigated soils affected by salinity (Zhu, 2001), a situation worsened by climate change

Abiotic stress leads to a series of morphological, physiological, biochemical, and molecular changes that adversely affect plant growth and productivity (Helaly, 2017). Drought, salinity, extreme temperatures, and oxidative stress are often interconnected, and may induce similar cellular damage (Jewell et al., 2010) .During the course of its evolution, plants have developed mechanisms to cope with and adapt to different types of abiotic and biotic stress. Plants face adverse environmental conditions by regulating specific sets of genes in response to stress signals, which vary depending on the factors such as severity of stress conditions, other environmental factors and the plant species (Wang et al., 2001)

The sensing of these stresses induces signalling events that activate ion channels, kinase cascades, production of reactive oxygen species, and accumulation of hormones (Cheong et al ., 2002). These signals ultimately induce expression of specific genes that lead to the assembly of the overall defence reaction. . In contrast to plant resistance to biotic stresses, which is mostly dependent on monogenic traits, the genetically complex responses to abiotic stresses are multigenic, and thus more difficult to control (Vinocur et al., 2005)

The most important strategies of plant crop improvements against stresses are conventional breeding method and tissue culture. The conventional breeding programs are being used to integrate genes of interest from intercrossing genera and species into the crops to induce stress tolerance. However, in many cases, these conventional breeding methods have failed to provide desirable results (Rai et al., 2011).

The conventional breeding programs like selection, hybridization, backcross breeding and composite crossing and multiline were used, but faced several problems such as slow, take long time, cost money and hectic for developing resistance in crops (Helaly, 2017). Further, classical breeding faced some difficulty affected the hybridization such as environmental conditions in particular, low and high relative humidity, as well as low and high temperature.

In recent years, the use of techniques based on *in vitro* plant tissue culture, has made possible the development of biotechnological tools for addressing the critical problems of crop improvement for sustainable agriculture. Among the available biotechnological tools for cropbreeding, genetic engineering based on introgression of genes that are known to be involved in plant stress response and *in vitro* selection through the application of selective pressure in culture conditions, for developing stress tolerant plants, have proved to be the most effective approaches (Helaly, 2017).

In vitro tissue culture-based tools have also allowed a deeper understanding of the physiology and biochemistry in plants cultured under adverse environmental conditions (Benderrdji et al., 2012). In this paper, the progress made towards the development of abiotic stress-tolerant plants through tissue culture-based approaches is described. The achievements in the better understanding of physiological and biochemical changes in plants under *in vitro* stress conditions are also reviewed.

2. Somaclonal variation

Plant improvement through somaclonal variation and *in vitro* is some techniques of *in vitro* culture for obtaining some plant genotype tolerant to the biotic or abiotic stresses (Yusnita et al., 2005). *In vitro* selection is one of somaclonal variation method. Somaclonal variation is defined as the genetic and phenotypic variation among clonally propagated plants of a single donor clone. It is well known that genetic variations occur in undifferentiated cells, isolated protoplasts, calli, tissues and morphological traits of regenerated plants. The cause of variation is mostly attributed to changes in the chromosome number and structure. Generally, the term somaclonal variation is used for genetic variability present among all kinds of cells/plants obtained from cells cultured *in vitro* (Lestari, 2006).

Plant regeneration from tissue and cell culture show heritable variation for both qualitative and quantitative traits. Somaclonal variation caused by the process of tissue culture is also called tissue culture induced variation. The occurrence of uncontrolled and spontaneous variation during the culture process is an unexpected and mostly undesired phenomenon when plants are micropropagated at commercial scale (Gao et al., 2010). However, apart from these negative effects, its usefulness in crop breeding through creation of novel variants has been extensively reported (Predierj, 2007). Induced somaclonal variation can be used for genetic manipulation of crops with polygenic traits (Jain, 2001). The new varieties derived from in vitro tissue culture could exhibit disease resistance and improvement in quality as well as better yield (Biswas et al., 2009).

Somaclonal variation occurs among the population of plant resulted from in vitro culture. It is apparently caused by gene amplification, the alteration of a basic couple, transposing migration, methylation transform, chromosome instability, chromosome inversion, one spot mutation, translocation, ploidy change and restructuring or deletion (Kumar and Murthur, 2004). Somaclonal variants can be detected using various techniques which are broadly categorized as morphological, physiological, biochemical and molecular detection techniques. There are two main approaches for isolation of somaclonal variants. These are screening method and cell selection method.

Screening involves the observation of a large number of cells or regenerated plants for the detection of variant individuals. Mutants for several traits can be far more easily isolated from cell cultures than from whole plant populations. This is because a large number of cells can be easily and effectively screened for mutant traits. Screening of as many plants would be very difficult, ordinarily impossible. Mutants can be effectively selected for disease resistance, improvement of nutritional quality, adaptation to stress conditions, e.g., saline, soils, low temperature, toxic metals, resistance to herbicides and to increase the biosynthesis of plant products used for medicinal or industrial purposes. Screening has been profitably and widely employed for the isolation of cell clones that produce higher quantities of certain biochemical (Matkowski, 2008).

In the cell selection approach, a suitable pressure is applied to permit the preferential Survival/growth of variant cells. Selection strategies have been successfully developed for the recovery of genotypes resistant to various toxins, herbicides, high salt concentration etc. (Zair et al., 2003). When the selection pressure allows only the mutant cells to survive or divide, it is called positive selection. On the other hand, in the case of negative selection, the wild type cells divide normally and therefore are killed by a counter selection agent, e.g., 5- Bromodeoxyuridine, or arsenate. The mutant cells are unable to divide as a result of which they escape the counter selection agent. These cells are subsequently rescued by removal of the counter selection agent (Rai et al., 2011).

3. *In vitro* selection of plants tolerant to abiotic stress

Many studies have reported that the *in vitro* culture alone or combined with mutagenesis, induced with physicochemical or biological agents, can be exploited to increase genetic variability and mutants, as a potential source of new commercial cultivars (Helaly, 2017). *In vitro* culture environments can be mutagenic and plants regenerated from organ cultures, calli, protoplasts and via somatic embryogenesis sometimes exhibit phenotypic and/or genotypic variations.

Table 1: *In vitro* selection for increased resistance to abiotic stresses

Plant species	stress	References
Chrysanthemum morifolium (chrysanthemum)	Salt	Hossain et al., 2007.
Brassica napus	Salt	Rahman et al., 1995.
Citrus aurantium	Salt	Koc et al., 2010.
Solanumlycopersicum	Salt	Kripkya et al., 2001.
Dendrocalamusstrictus	Salt	Rai et al., 2012.
Ipomoea batatas	Salt	He et al., 2009
Saccharum sp.	Salt	Gondonov et al., 2006.
Solanumtuberosum	Salt	Ochatt et al., 1999.
Arachishypogaea	Drought	Purushothman et al., 1998.
Brassica juncea	Drought	Gangopadhyay et al., 1997.
Prunusavium	Drought	Ochatt et al., 1999.
Saccharumsp.	Drought	Errabii et al., 2006
Triticumaestivum	Drought	Barakat et al., 1996.
Glycine max	Drought	Mariska, 2003.

The most widely used method for the selection of genotypes tolerant to abiotic stress is the *in vitro* selection pressure technique. This is based on the *in vitro* culture of plant cells, tissues or organs on a medium supplemented with selective agents, allowing selecting and regenerating plants with desirable characteristics. In table 1 a list of species in which this technique has been successfully applied to obtain genotypes with increased resistance to different abiotic stresses is shown.

3.1. *In vitro* selection of salt-tolerant plants

The problem of soil salinity has been aggravated during the last decades as a consequence of some agricultural practices such as irrigation and poor drainage systems. It has been estimated that around 20 % of the irrigated land in the world is affected by salinity, and it is expected that the increase of salinization in agricultural fields will reduce the land

available for cultivation by 30% in the next 25 years and up to 50% by the year 2050 (Rozema and Flowers, 2008).

The *in vitro* selection pressure technique has been effectively utilized to induce tolerance to salt stress in plants through the use of salts as a selective agent, allowing the preferential survival and growth of desired genotypes. This approach has being done using a number of plant materials (callus, suspension cultures, somatic embryos, shoot cultures, etc.) which has been screened for variation in their ability to tolerate relatively high levels of salt in the culture media. In most of the studies, the salt used has been NaCl. (Woodward and Bennet, 2005).

3.2. *In vitro* selection of drought-tolerant plants

Drought is a major abiotic stress which causes important agricultural losses, mainly in arid and semiarid areas. Drought stress causes moisture depletion in soil and water deficit with a decrease of water potential in plant tissues. *In vitro* culture has been used to obtain drought tolerant plants assuming that there is a correlation between cellular and *in vivo* plant responses Mohamed et al., 2000). During the last years, *in vitro* selection for cells exhibiting increased tolerance to water or drought stress has been reported (Table 1).Polyethylene glycol (PEG), sucrose, mannitol or sorbitol have been used by several workers as osmotic stress agents for *in vitro* selection (Hassan et al., 2009).

However, PEG has been the most extensively used to stimulate water stress in plants. This compound of high molecular weight is a non-penetrating inert osmoticum that reduces water potential of nutrient solutions without being taken up by the plant or being phytotoxic (Hassan et al., 2005). Because PEG does not enter the apoplast, water is withdrawn not only from the cell but also from the cell wall. Therefore, PEG solutions mimic dry soil more closely than solutions of compounds with low molecular weights, which infiltrate the cell wall with solute (Verslues et al., 1998).Besides salt and drought, a few reports are also available for the development of plants tolerant to other abiotic stress (metal, chilling, UV and frost) through *in vitro* selection. Besides salt and drought, a few reports are also available for the development of plants tolerant to other abiotic stress (metal, chilling, UV and frost) through *in vitro* selection.

4. Transgenesis for abiotic stress tolerance

Transgenic approaches are among the available tools for plant improvement programs based on biotechnological methodologies. Nowadays, many mechanisms and gene families, which confer improved productivity and adaptation to abiotic stresses, are known. These gene families can be manipulated into novel combinations, expressed ectopically, or transferred to species in which they do not naturally occur. Therefore, the possibility to transform the major crop species with genes from any biological source (plant, animal, microbial) is an extremely powerful tool for molecular plant breeding (Helaly, 2017).

To date, successes in genetic improvement of environmental stress resistance have involved manipulation of a single or a few genes involved in signalling/regulatory pathways or that encode enzymes involved in these pathways (such as osmolytes/compatible solutes, antioxidants, molecular chaperones/osmoprotectants, and water and ion transporters. The disadvantage of this approach is that there are numerous interacting genes involved, and efforts to improve crop drought tolerance through manipulation of one or a few of themis often associated with other, often undesirable, pleiotropic and phenotypic alterations (Wang et al., 2012).

The plant hormone abscisic acid (ABA) regulates the adaptive response of plants to environmental stresses such as drought, salinity, and chilling via diverse physiological and developmental processes. The ABA biosynthetic pathway has been deeply studied and many of the key enzymes involved in ABA synthesis have been used in transgenic plants in relation to improving abiotic stress tolerance (Schwartz et al., 2014). Transgenic plants over expressing the genes involved in ABA synthesis showed increased tolerance to drought and salinity stress. Similarly, many studies have illustrated the potential of manipulating CBF/DREB genes to confer improved drought tolerance (Trujillo et al., 2012)

Another mechanism involved in plant protection to osmotic stress associated to drought and salinity involves the up regulation of compatible solutes that function primarily to maintain cell turgor, but are also involved in avoiding oxidative damage and chaperoning through direct stabilization of membranes and/or proteins (Zang et al., 2012). Many genes involved in the synthesis of these osmoprotectants have been explored for their potential in engineering plant abiotic stress tolerance. The cellular and metabolic processes involved in salt stress are similar to those occurring indrought-affected plants and are responses to the osmotic effect of salt (Muns and Tester 2008). As described above, the use of genes related to osmoprotectant synthesis has been successfully used in developing drought-tolerant crops and transfer of glycine betaine intermediates developing drought-tolerant crops and the transfer of glycine betaine intermediates have improved the drought and salt tolerance of transgenic plants in many cases. The amino acid proline is known to occur widely in higher plants and normally accumulates in large quantities in response to environmental stresses (Kishore et al., 2005). The osmoprotectant role of proline has been verified in some crops by over expressing genes involved in proline synthesis (Himida-Sayari et al., 2005). Other approaches successfully developed in a variety of crops to obtain abiotic-stress tolerant plants by transgenesis, have been manipulation of transcription factors (TFs), lateembryogenesis abundant (LEA) proteins, and antioxidant proteins (Umezawa et al., 2012).On the other hand, the use of genetic and genomic analysis to identify DNA molecular markers associated to stress resistance can facilitate breeding strategies for crop improvement. This approach is particularly useful when targeting characters controlled by several genes, as in the case of most abiotic stress.

The potential to map different Quantitative Trait Loci (QTL) contributing to an agronomicaltrait and to identify linked molecular markers opens up the possibility to transfer.

Simultaneously several QTLs and to pyramid QTLs for several agronomical traits in one improved cultivar. However, the application of molecular markers in breeding programs requires preliminary studies to identify and validate potential markers. QTLs and to pyramid QTLs for several agronomical traits in one improved cultivar. However, the application of molecular markers in breeding programs requires preliminary studies to identify and validate potential markers (Yu et al., 2004).

Although the use of Marker-Assisted Selection may be helpful for crop improvement, its practical application in genetic improvement of resistance or tolerance to stress has been limited since no many stress tolerance QTL have been identified [56]. For future biotechnology improvements such as tolerance to drought or nutrient limitation, forward breeding will be necessary to co-optimize transgenic expression and genetic background because endogenous genes and environmental factors may have the potential to influence the phenotypes resulting from transgenic modifications (Mumm, 2007).

It is important to point that genetic modification of higher plants by introducing DNA into their cells is a highly complex process. Practically any plant transformation experiment relies at some point on cell and tissue culture. Although the development transformation method that avoid plant tissue culture have been described for *Arabidopsis*, and have been extended to a few crops, the ability to regenerate plants from isolated cells or tissues *in vitro* is needed for most plant transformation systems. Not all plant tissue is suited to every plant. transformation method, and not all plant species can be regenerated by every method (Bensoo, 2000).There is, therefore, a need to find both a suitable plant tissue culture/regeneration regime and a compatible plant transformation methodology.

5. *In vitro* tissue culture as a tool for physiological and biochemical studies in plants

Because of the great interest for both basic and applied research, many scientific endeavours have long addressed the understanding of the mechanisms underlying the stress response and the identification of the specific genes/metabolites that are responsible for tolerance phenotypes.

In the last decades, *in vitro* culture of plants has become an integral part of advances in plant science research. Plant tissue culture techniques allow for close monitoring and precise manipulation of plant growth and development, indeed, the *in vitro* system offers the advantage that relatively little space is needed to culture plants and this system allows a rigorous control of physical environment and nutrient status parameters, which are difficult to regulate with traditional experimental system. Furthermore, any complex organorgan and plant-environment interaction can be controlled or removed, and the level of stress can be accurately and conveniently controlled (Stephen et al., 2002). All this together makes that some aspects of plant growth, that were barely understood before the advancement of the science of tissue culture, such as the metabolism and interaction of plant hormones, as well as their physiological effects can be deeply studied (Bairu and Kane, 2011).

Shoot apex culture has been widely used to evaluate plant physiological responses to salinity and osmotic stress in various species, including apple, olive and tomato. With regard to the whole plant, a similar response to salt stress could be expected in plantlets grown through *in vitro* shoot apex culture, because such explants can be considered mini-replicas of a plant with anatomical organization and ability to root and grow into whole plant (Cano et al., 1998).

It has been previously described the use of an *in vitro* tissue culture technique to study the performance of different citrus genotypes cultured under salt stress conditions, avoiding the effect of the root by culturing shoots without the root system. The method proved to be a good tool for studying biochemical processes involved in the response of citrus to salt stress. Some citrus genotypes have been classified as relatively salt tolerant under field conditions due to their ability to restrict chloride ions to roots while others have proved to be more sensitive to salinity (Lopez et al., 2008).

In vitro tissue culture approach allowed us to observe that when shoots are cultured without a root system, all genotypes accumulated the same chloride levels and exhibited similar leaf damage as a consequence of salt stress treatment. There was no increase of malonyldialdehyde levels in any genotype, and common patterns of hormonal signaling were observed among genotypes. On the view of these results we concluded that under the same salt conditions and with the same level of leaf chloride intoxication, no biochemical differences exist among tolerant and sensitive genotypes. This points to the roots as a key organ not only as a filter of chloride ions but also as a signalling system in citrus (Montoliu et al., 2009).In vitro tissue culture provided the tools to perform this studies that it would be impossible to carry out with whole plants grown under field or greenhouse conditions.

6. Conclusion

Use of *in vitro* cell and tissue-based systems offers a remarkable tool for dissecting the physiological, biochemical and molecular regulation of plant development and stress response phenomena. In recent years, considerable progress has been made regarding the development and isolation of stress tolerant genotypes by using *in vitro* techniques. The most successful applied tools have been the induction of somaclonal variation and *invitro* selection of plants tolerant to different abiotic stresses and the development of transgenic genotypes throughout different approaches

Development of transgenic plants using biotechnological tools has become important inplant-stress biology. Previous works on genetics and molecular approaches have shown that most of the abiotic stress tolerant traits are multigenic. Therefore, to improve stress tolerance several stress related genes need to be transferred. More recently manipulation of single transcription factors has provide the same effect as

manipulation of multiple genes. This has become a promising approach to get abiotic stress tolerant crops.

In vitro selection makes possible to save the time required for developing abiotic stress tolerant lines of commercial crops and other plant species. However, i*nvitro* selected variants should be finally field-tested to confirm the genetic stability of the selected traits under field conditions. Our knowledge about the molecular mechanisms operational during stress tolerance under invitro conditions in plant is also limited. The development of *in vitro* selection technology, together with molecular approaches and functional genomics will provide a new opportunity to improve stress tolerance in plants relevant to food production and environmental sustainability.

References

[1] Arbona V, Gómez-Cadenas A., 2008. Hormonal modulation of citrus responses to flooding. Journal of Plant Growth Regulation, 27 241-250.
[2] Ashraf M., Foolad M.R., 2007, Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environmental and Experimental Botany; 59 206–216.
[3] Bairu M.W., Kane M.E., 2011, Physiological and developmental problems encountered by *in vitro*cultured plants. Plant Growth Regulation, 63 101–103
[4] Benderradji L., Brini F., Kellou K., Ykhelf N., Djekoun A., Masmoudi K., Bouzerour. H., 2012, Callus induction, proliferation, and plantlets regeneration of two bread wheat (*Triticum aestivum*L.) genotypes under saline and heat stress conditions. ISRN Agronomy, Article ID 367851.
[5] Benson E.E., 2008, Special symposium: *in vitro* plant recalcitrance *In vitro* plant recalcitrance: an introduction. *In Vitro* Cellular and Developmental Biology Plant, 36:141–148.
[6] Cano E.A., Perez-Alfocea F., Moreno V., Caro M., Bolarin, M.C., 1998, Evaluation of salt tolerance in cultivated and wild tomato species through *in vitro* shoot apex culture. Plant Cell, Tissue and Organ Culture, 53 19-26.
[7] Cheong Y.H., Chang H.S., Gupta R., Wang X., Zhu T., Luan S., 2002, Transcriptional profiling reveals novel interactions between wounding, pathogen, abiotic stress, and hormonal responses in Arabidopsis. Plant Physiology, 129: 661–677.
[8] Errabii T., Gandonou C.B., Essalmani H., Abrini J., Idomar M., Senhaji N.S., 2006, Growth, proline and ion accumulation in sugarcane callus cultures under drought-induced osmotic stress and its subsequent relief. African Journal of Biotechnology. 5 1488- 1493.
[9] Gandonou C.B., Errabii T., Abrini J., Idaomar M., Senhaji N.S., 2006, Selection of callus cultures of sugarcane (*Saccharum*sp.) tolerant to NaCl and their response to salt stress. Plant Cell Tissue and Organ Culture. 87 9-16.
[10] Gangopadhyay G., Basu S., Gupta S., 1997, *In vitro* selection and physiological characterization of NaCl- and mannitol-adapted callus lines in *Brassica juncea*. Plant Cell Tissue and Organ Culture., 50 161-169
[11] Gao X., Yang D., Cao D., Ao M., Sui X., Wang Q., Kimatu J., Wang L., 2010, *In vitro* micropropagation of Freesia hybrida and the assessment of genetic and epigenetic stability in regenerated plantlets. Journal of Plant Growth Regulation, 29 257–267.
[12] Hassan N.M., Serag M.S., El-Feky F.M., 2004, Changes in nitrogen content and protein profiles following*in vitro* selection of NaCl resistant mung bean and tomato. ActaPhysiologiae Plantarum, 26 165-175.
[13] He S., Han Y., Wang Y., Zhai H., Liu Q., 2009, *In vitro* selection and identification of sweet potato (*Ipomoea batatas*(L.) Lam.) plants tolerant to NaCl. Plant Cell Tissue and Organ Culture, 96: 69-74.
[14] Helaly AA, . 2017/ Strategies for Improvement of Horticultural Crops against Abiotic Stresses. J Hortic 4:e107. doi: 10.4172/2376-0354.1000e107
[15] Hmida-Sayari A., Gargouri-Bouzid R., Bidani A., Jaoua L., Savoure A., Jaoua S., 2005, Overexpression of D1-pyrroline-5-carboxylate synthetase increases proline production and confers salt tolerance in transgenic potato plants. Plant Science, 169 746–752.
[16] Hossain Z., Mandal A.K.A., Datta S.K., Biswas A.K., 2007, Development of NaCl tolerant line in *Chrysanthemum morifolium*Ramat. through shoot organogenesis of selected callus line. Journal of Biotechnology., 129 658-667.
[17] Jain S.M., 2001, Tissue culture-derived variation in crop improvement. Euphytica, 118, 153–166.
[18] Jewell M.C., Campbell B.C, Godwin I.D., 2010, transgenic plants for abiotic stress resistance. In: Transgenic Crop Plants. C. Kole et al. (eds.), pringer-Verlag Berlin Heidelberg.
[19] Kavi Kishore P.B., Sangam S., Amrutha R.N., Laxmi P.S., Naidu K.R., Rao K.R.S.S., Rao S., Reddy K.J., Theriappan P., Sreenivasulu N., 2005, Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: its implications in plant growth and abiotic stress tolerance. Current Science, 88 424–438.
[20] Koc N.K., Bas B., Koc M., Kusek M., 2009, Investigations of *in vitro* selection for salt tolerant lines in sour orange (*Citrus aurantium*L.). Biotechnology, 8 155-159.
[21] López-Climent M.F., Arbona V., Pérez-Clemente R.M., Gómez-Cadenas A., 2008, Relationship between salt tolerance and photosynthetic machinery performance in citrus. Environmental and Experimental Botany, 62 176–184.
[22] Mariska I., 2012, PeningkatanKetahananterhadapAlumuniumpadaPertanaanKedelai melaluiKultur in Vitro. Laporan RUT VIII. BidangTeknologiPertanian. Bogor: Balai PenelitianBioteknologiPertanian&KementerianRisetdan Teknologi RI-LIPI.
[23] Matkowski A., 2008, Plant*in vitro* culture for the production of antioxidants — A review. Biotechnology Advances, 26 584-560.
[24] Mohamed M.A.H., Harris, P.J.C., Henderson, J., 2000, *In vitro* selection and charac-terisation of a drought tolerant clone of *Tagetesminuta*. Plant Science, 159 213-222.
[25] Montoliu A., López-Climent M.F., Arbona V., Pérez-Clemente R.M., Gómez-Cadenas, 2009, A novel *in vitro*

tissue culture approach to study salt stress responses in citrus. Plant Growth Regulation, 59 179-187.
[26] Mumm R.H., 2007, Backcross versus forward breeding in the development of transgenic maize hybrids: theory and practice. Crop Science *(Suppl 3)*, 47: 164–171.
[27] Munns R., Tester M., 2008, Mechanisms of salinity tolerance. Annual Review of Plant Physiology, 59 651–681.
[28] Ochatt S.J., Marconi P.L., Radice S., Arnozis P.A., Caso O.H., 1999, *In vitro* recurrent selection of potato: production and characterization of salt tolerant cell lines and plants. Plant Cell Tissue and Organ Culture, 55 1-8.
[29] Predieri S., 2001, Mutation induction and tissue culture in improving fruits. Plant Cell Tissue and Organ Culture, 64 185–210.
[30] Purushotham M.G., Patil V., Raddey P.C., Prasad T.G., Vajranabhaiah S.N., 1998 Development of *in vitro* PEG stress tolerant cell lines in two groundnut (*Arachishypogaea* L.) genotypes. Indian Journal of Plant Physiology, 3 49-51.
[31] Rahman, M.H., Krishnaraj, S., Thorpe, T.A., 1995, Selection for salt tolerance *in vitro* using microspore-derived embryos of *Brassica napus*cv. Topas, and the characterization of putative tolerant plants. In Vitro Cellular and Developmental Biology - Plant, 31 116-121.
[32] Rai M.K., Kalia R .K., Singh R., Gangola M.P., Dhawan A.K., 2011, Developing stress tolerant plants through in vitro selection—An overview of the recent progress. Environmental and Experimental Botany, 71 89-98.
[33] Rozema J., Flowers T., 2008, Crops for a salinized world. Science, 322 1478-1480.
[34] Schwartz S.H., Qin X., Zeevaart J.A.D., 2003, Elucidation of the indirect pathway of abscisic acid biosynthesis by mutants, genes, and enzymes. Plant Physiology, 131 1591– 1601.
[35] Shibli R.A., Smith M.A.L., Spomer L.A., 1992, Osmotic adjustment and growth responses of three*Chrysanthemum morifolium*Ramat cultivars to osmotic stress induced *in vitro*. Journal of Plant Nutrition,, 15 1374-1381.
[36] Stephen R.G., Zeng L., Shannon M.C., 2002, Roberts S.R. Rice is more sensitive to salinity than previously thought. Annual Report of U.S. Department of Agriculture's Research Service, pp: 481.
[37] Trujillo L.E., Sotolongo M., Menendez C., Ochogavia M.E., Coll Y., Hernandez I., Borras-Hidalgo O., Thomma B.P.H.J., Vera P., Hernandez L., 2008, SodERF3, a novel sugarcane ethylene responsive factor (ERF), enhances salt and drought tolerance when overexpressed in tobacco plants. Plant and Cell Physiology, 49 512–525.
[38] Umezawa T., Fujita M., Fujita Y., Yamaguchi-Shinozaki K., Shinozaki K., Engineering Vinocur B., Altman A, 2005. Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. Current Opinion in Biotechnology, 16 123-132.
[39] Wang W., Vinocur B., Altman A., 2003, Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta, 218 1–14.
[40] Yang Y.L., Shi R.X., Wei X.L., Fan Q., An L.Z., 2010, Effect of salinity on antioxidant enzymes in calli of the halophyte *Nitrariatangutorum* Bobr. Plant Cell Tissue and Organ Culture, 102 387–395.
[41] Zhang Y.Y., Li Y., Gao T., Zhu H., Wang D.J., Zhang H.W., Ning Y.S., Liu L.J., Wu Y.R., Chu C.C., Guo H.S., Xie Q., 2008, Arabidopsis SDIR1 enhances drought tolerance in crop plants. Biosci Biotechnol Biochem 72: 2251–2254.