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Role of proline in plant stress tolerance : A mini review

ARSHAD NAJI ALHASNAWI*

Department of Biology
College of Education for Pure Sciences
Al Muthanna University, Al Muthanna, Samawah 66001, Iraq
**(e-mail : arshad@mu.edu.iq)*

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ABSTRACT

Abiotic stresses assess the main impendences or barriers to the improvement of agriculture. Subsequently, the efforts to improve stress-tolerant plants are of major importance in increasing crop productivity. In recent years, plant tissue culture techniques established *in vitro* have appeared as cost-effective tools and a practical method for the development of stress tolerance in plants. The relatively powerful tolerance of plant cultivar to salinity was also related to the capability of plants to accumulate increased levels of proline. The adjustment of the accumulation of proline was estimated in sodium-chloride-adapted versions of a salt-sensitive and a salt-tolerant plant, respectively, following sodium chloride shock, and defined a biotechnology technique for the enhancement of salt tolerance in crops.

Key words : Abiotic stress, biotic stress, proline, tolerance

INTRODUCTION

In biotechnology, *in vitro* techniques can be perfect options for utilizing cells or plant tissues for performance of salt stress study, which has the ability to unravel halophyte plants, develop a salinity-tolerant technique at the organized tissue stage, or messy cellular. Such can also provide information on the possibility for biochemical, physiological and improvement responses to salinity stress at numerous levels of tissue association. Furthermore, plant tissue culture techniques realization permits relatively shorter group times, more precise settings and faster replies as compared with *ex vitro* circumstances (Perez-Tornero *et al.,* 2009).

Proline is a major osmolyte that accumulates under a NaCl stress medium in different plants and in the context of adaptation mechanisms to water shortage and salinity (Kumar *et al.,* 2003). Proline content as an osmoregulation increases with the stress of sodium chloride (NaCl) (Alhasnawi *et al.,* 2016). Vinocur and Altman (2005) observed that proline collected (usually in the cytosol) through saline conditions and was associated with osmotic change for enhancing plants' salt tolerance. The present review represents a pioneering assistance to the overall repository in tissue culture and an important accumulation of proline in salt tolerance.

Propagation *in vitro*

It has been reported that *in vitro* methods are useful to breeders in generating new genotypes of crops. Gai *et al.* (2011) found that *in vitro* cultures of plants had been customarily used by plant breeders for several years and have led to the introduction of several genotypes that are currently grown and eaten as food all over the world. Largely, the new genotypes had grown well in farmers' fields and have not affected human health. Larkin (2004), Dan *et al.* (2010) also reported that the number of genetic differences that occur as an outcome of *in vitro* culturing was manageable and mild. Still, they happen regularly and can be very either detrimental or positive in the expression of the rate of the crops. It is thus the job of a plant breeder to select favourable combinations of traits and eliminate damaged mutations. To this end, the concept of somaclonal difference is an important technique in plant breeding.

Gamborg and Phillips (1995) noted that the *in vitro* technique of plants was mainly dependent upon the composition of the plant

media. *In vitro* induction medium is mainly composed of sugar, carbon, mineral salts and water. Though the magnitude of the several ingredients in the media differs with stages of culture and plant genotypes, the requisite MS media (Murashige and Skoog, 1962) as well as LS media (Linsmaier and Skoog, 1965) remain the typically used medium types. The methods readily used for propagating plants *in vitro* are similar to those previously developed for conventional propagation. Research (George and Debergh, 2008) has found that the use of *in vitro* methods has subsequent benefits over traditional methods, as follows :

1. Cultures are initiated with small parts of a plant and, subsequently, shoots or embryos are propagated (hence the use of the term 'micropropagation' to characterize the *in vitro* methods). Just a small amount of space is required to maintain plants or to multiply them. Propagation is generally carried out in aseptic conditions (to avert contamination).
2. The most flexible modification of factors responsible for vegetative growth is possible e. g. growth regulator and nutrient levels, temperature and light. As a result, the average of propagation is higher than that in micropropagation and a large number of plants can be produced within a given period.
3. Approaches are available to rid plants of certain viral diseases. However, terminologies for example bacteria-free and virus-free should not be used, as no investigator has been able to show definitively that a plant is resistant against all viruses or bacteria.
4. Production can be more independent of seasonal changes and is continued all year-round.
5. A lesser amount of space and energy is essential for micropropagation technique.
6. The process may produce clones of some species of plants that are impossible or slow to propagate vegetatively.
7. Plants possibly acquire newer short-term characteristics during micropropagation.
8. Vegetatively-reproduced substances can be stored for longer periods.
9. Micropropagation can show an advantage if it costs less than traditional techniques of multiplication; otherwise, there needs to be other very important reasons in order to make it worthwhile.

Basics of Tissue Culturing and Plant Cells

As already discussed in previous sections, tissue culture techniques are utilized in a pathogen-free manner for the micropropagation and production of plants (Kaviani *et al.,* 2011). Sharma and Agrawal (2012) reported that *in vitro* tissue and a cell-based system had enormous potential in important studies and for technology commercial applications for example genetic engineering, the production of useful secondary metabolites and clonal propagation.

The commercial applications are primarily based on the principle of micropropagation, in which rapid proliferation is achieved from axillary buds, small stem cuttings, somatic embryos and cell clumps (Ahloowalia *et al.,* 2004). It also highlights the efficiency of a single cell to reflect the full genome by cell division. This, together with the totipotent potential of a plant cell as well as the ability of plant cells to develop and alter their metabolism, is equally important and crucial in regenerating the complete plant (Thorpe, 2007). Murashige and Skoog (1962) showed that, similarly to in the case of natural soil and aquatic ecosystems, plant tissue culture media included each nutrient required for the development and normal growth of plants. It is important composed of vitamins, micronutrients, macronutrients and organic components, a carbon source, and gelling agents, in the case of a solid media and hormones.

Another essential term in plant tissue culture technique is callus; this is an amorphous and coherent tissue that is created when plant cells multiply in a disorderly manner. Calli can be started *in vitro* by explants

input into a growth-supporting media under sterile environments (George, 2008). The term 'media' refers to that which can accommodate the growth of plant material *in vitro* and contains specialised nutrients. It typically consists of a salts solution that can supply both the primary and more minor elements essential for the development of whole plants, along with amino acids and various vitamins in some cases as well as an energy source (e. g. sucrose) (George, 2008).

Proline in Abiotic Stress

It is clear that, among the diverse characteristics responding to salt stress, quick increase of proline in the cell is the generality considerable variation one that will prompt an increase in plant salt tolerance (Alhasnawi *et al.,* 2017). With an enhancement in sodium chloride stress, the endogenous free proline concentrations of plants also increase (Ketchum *et al.,* 1991). Schat *et al.* (1997) also found that, in *Silene vulgaris*, essential amino acid proline levels were increased in metal-tolerant ecotypes, whereas in the case of metal accumulation, amino acid proline increase was higher in a non-tolerant ecotype. Notably, the relationship between abiotic stress tolerance and free proline increase in plants is not constantly visible. The high levels of proline can be distinctive of the salt and cold-hypersensitive plant *Arabidopsis* (Xin and Browse, 1998). The accumulation of proline in plants can be mediated by both ABA-independent and ABA-dependent signalling pathways (Zhu, 2002). Hasegawa *et al.* (2000) suggested that, when the plant is under saline conditions, they respond with an increased accumulation of agreeability solutes such as proline in the cytosol, which improves the damage effects of salt stress.

As a result of the above, a low content of glycine betaine and proline against optimum levels of soluble sugar accumulation and sodium ions at a concentration of 200 mM of sodium chloride stress might transfer a lot of energy for the improvement of shoot growth and the maintenance of homeostasis. Moreover, the NaCl might occasionally elicit positive protein conformational alterations, which can serve as an indication to initiate the signal transduction cascade into the NaCl adaptation mechanism in plants challenged to sodium chloride (200 mM) (Yang and Yen, 2002).

High proline levels in the halophyte plant *Pancratium maritimum* can increase the salt tolerance by protein turnover, the stabilization of detoxifying enzymes, and the stimulation of the accumulation of stress protection of proteins (Khedr *et al.* 2003). In contrast, Rahnama and Ebrahimzadeh (2004) performed a study involving potato seedlings and found no clear relationship between salt tolerance and the accumulation of proline. Niknam *et al.* (2004) also reported the effects of NaCl on *in vitro* growth parameters in addition to proline, proteins and sugars in the leaf explants and seedlings of *Nicotiana tabacum*. In the seedlings, dry and fresh weight values were reduced under saline conditions. Proline accumulation in leaf and seedlings of explants improved, but the content of the polysaccharide reduced with a rise in the levels of NaCl. It has been suggested that proline may play a crucial role in preventing osmotic stress.

Proline usually accumulates in cytosol under saline conditions and is correlated with osmotic adjustment to improve the adaptation of a plant to saline conditions (Vinocur and Altman, 2005). When seeds were subjected to germination on media with NaCl stress, it was found that tomato *Lycopersicon esculentum* accumulated proline in the leaf and stem to a degree that is much higher than in the roots in all cultivars (Amini and Ehsanpour, 2005). Separately, Velfisquez *et al.* (2005) reported no association between salt tolerance and proline increase among different potato cultivars, in spite of the considerable variation that was noted among these genotypes during *in vitro* screening.

Kant *et al.* (2006) observed an increase in the accumulation of proline in salt-tolerant calli versus in non-selected calli of sugarcane (Gandonou *et al.,* 2006). Niknam *et al.* (2006) noted the effects of NaCl on protein, proline value, growth, and polyphenol oxidase, peroxidase and catalase activities in the seedlings and callus cultures of fenugreek. Seeds and hypocotyl explants were cultured on the media of MS added with sodium chloride.

Seed germination as well as fresh and dry mass values of the seedlings were reduced with saline conditions. In both the species, an increase in the content of protein in the seedlings was found under NaCl stress. Furthermore, the NaCl provoked changes in the accumulation of polyphenol oxidase, catalase and peroxidase in calli and seedlings (Demiral and Türkan, 2006). It has additionally been described that salinity stress greatly enhanced the proline increase in the leaves of rice cultivars, albeit differing in the salt tolerant versions, and that the rate of increase was a lot higher in the tolerant one, suggesting that the accumulation of proline contributes to osmotic modification through salt conditions. Molinari *et al.* (2007) observed in the stress-induced accumulation of proline in transgenic sugarcane plants that water-deficit stress acted as a factor of an antioxidative protection system, sort of like an osmotic modification regulator.

Another example of proline accumulation in purslane *Portulaca oleracea* L. plants may represent an adaptive feature to protect the plant's water balance in short-term saline conditions persuaded by osmotic stress (Yazici *et al.,* 2007). However, the increased proline was not enough to maintain the water balance of purslane at the next long-term exposure to salinity stress. Conversely, other halophytes, for example *Limonium* spp. or *Camphorosma annua*, usually do not have high proline levels; when such begins to accumulate, they increase their betain-derived osmolyte or carbohydrate levels in retaliation (Murakeözy *et al.,* 2003; Gagneul *et al.,* 2007). Proline accumulation in response to NaCl stress changed with both the salinity condition and the absence or presence of auxin in calli (Patade and Suprasanna, 2009).

Investigators using mutants or transgenic plants were able to demonstrate that amino acid proline metabolism had a complex impact on stress responses and development and that the increase of proline was necessary for the plant tolerance of specific adverse abiotic stressors (Miller *et al.,* 2009).

Other studies have also found that the levels of proline in root exudates and root tissues from all plants increased following the rise in the concentration of salt in the medium, a trend closely linked with that of the entire tissue of the plant. In the plant, the role of proline accumulates and its responses to saline conditions could be studied by using non-destructive techniques. Furthermore, proline accumulates could be beneficial for the early discovery of salinity tolerance, as long as an association between salt stress tolerance and proline exists (Marin *et al.,* 2010; Alhasnawi *et al.,* 2014).

The contents of the proline in gerbera, *Gerbera jamesoniil* in leaves and roots are heightened, affected by increasing salt levels (Ganege Don *et al.,* 2010). On the other hand, the accumulation of glycine betaine and free proline in the axillary shoots of plant (*Sesuvium portulacastrum*) was considerable variation decreased at a salt stress level of 200 mM, while a progressive accumulation was found with an accumulate in the salinity to between 400 to 600 mM as compared with in the control (Lokhande *et al.* 2011).

Kang *et al.* (2012) observed an accumulation of proline patterns of *Ailanthus altissima* calli in response to salinity at NaCl concentrations of 0 to 2.0% in the basal MS liquid medium. Under NaCl, the free medium showed a decrease in proline concentration. Meanwhile, considering the calli cultured in medium containing a salt-induced accumulation of proline, the proline accumulations showed a linear elevated with increased levels of concentrations of NaCl in the medium for growth, with 1.0% of NaCl being effective.

CONCLUSION

The presented review suggests a conclusion that the provision of an amino acid proline source in plants until salt stress. The tissue culture technique represents a perfect method for utilizing cells or plant tissues for studies on the effectiveness of saline conditions as well as which efficiency is needed to unravel the halophyte plant, the use of saline tolerance techniques at the organized tissue stage, or messy cellular, and it can furthermore deliver information on the possibility for biochemical growth, and improvement replies to saline conditions at numerous levels of tissue association. Amino acid proline acts as an

antioxidant protection mechanism, helping the cells to avoid injury due to reactive oxygen species. Proline acts as a membrane protector, stabilizing proteins and membrane structures. Measurements in biotechnology such as amino acid proline accumulation contribute to osmotic adjustment through salinity. The modification of the accumulation of proline was estimated in a salt-sensitive plant and salt-tolerant plant after a salt shock and defines a biotechnology technique for enhancing and increasing the salt tolerance of crops.

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