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Plant Tissue Culture and Proline Accumulation in Abiotic Stress: A Review

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ABSTRACT

The term plant cell and tissue culture, which is also referred to as in vitro or artificial bioassay, and has gained wide acceptance both in scientific studies and commercial application. The success of plant biotechnology depends on the principles of plant tissue culture; which is an assembly of techniques used for the preservation or growth of plant cells, tissues or organs under sterile conditions in a nutrient culture medium of known composition. Additionally, plant tissue culture is used to produce clones of plant, a method renowned as micropropagation. On the other hand, Proline accumulation refers to a physiological response in plants resulting from a broad range of abiotic and biotic stresses. Nevertheless, there has been some debate as to the possible role of proline accumulation. This review, takes into consideration plant tissue culture as a technique used for in vitro regeneration of plants in the laboratory and how regulation of proline and high proline levels can improve the abiotic tolerance.

KEY WORDS: Plant Tissue Culture, Abiotic Stress, Proline Accumulation.

1. INTRODUCTION

The field of plant tissue culture is a multi-displinary area that offers interesting opportunities for crop production and quality enrichment (Jain, 2001). As a nascent tool, developed for rapid plant propagation, plant tissue culture has impacted greatly in the field of agriculture. In addition to rapid propagation, other advantages of this technology include; production of planting materials devoid of diseases as well as capacity for wider application e.g. crops, forest and fruit trees (Savangikar, 2004).

Also, plant tissue culture can be defined as a process involving the growth and multiplication of plant cells, tissues and organs on a specific solid or liquid sterile media under laboratory conditions. From a commercial point of view, the technology is based on micropropagation, whereby rapid growth and reproduction is accomplished from little stem cuttings, axillary buds, and to some extent from cell clumps in suspension cultures, somatic embryos and bioreactors (Ahloowalia et al., 2004).

There are various forms of plant tissue culture, (1) organ culture; is a general term for those types of culture whereby an organised form of growth can be unimpeded. It includes the aseptic separation from entire plants of its component parts such as leaf primordia, immature flowers and fruits, and their growth in the laboratory. When considering plant propagation, the most relevant kinds of organ culture are: meristem, shoot tip, shoot and bud culures respectively. Other organ cultures include; node cultures of separate lateral buds, each supported on a small piece of stem tissue; stem pieces, isolated root cultures and embryo cultures. (2) cultures of unorganized tissues; in this case, 'Tissue culture' is used as a combined term to represent all kinds of laboratoryplant cultures, even though it should precisely be used to refer to only cultures of unorganised aggregates of cells. In reality, the following kinds of cultures are mainly applicable: callus (or tissue) cultures, suspension (or cell) cultures, anther cultures and protoplast cultures (George, 2008).

Several investigators have attempted to produce abiotic tolerant plants utilizing tissue culture. This includes using a number of systems (i.e. shoot culture, suspension culture and callus) to screen for tissues and cells that exhibit differences in their capability to tolerate also endure high levels of salt (NaCl) in a growth media. To this end, some investigators have intensified efforts on agricultural species with some initial success in plants (Winicov, 1991; Johnson and Smith, 1992).

The use of cell and tissue cultures, aims at an improved comprehension of physiological, biochemical and anatomical reactions of specific cell material to certain factors under laboratory conditions, with the desire to gain insight into the life of the unaltered plant also in its natural environment. When compared to the use of unaltered plants, the major advantage of the cell tissue culture is that it is easier to control the physicochemical and environmental factors which are to be kept constant at affordable costs. It involves studying the growth and development of different plant parts without the influence of remote

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material in the unaltered plant body. In majority of instances, however, the original histology of the cultured material will undergo alterations, and may be lost in the process (Neumann et al., 2009).

In contrast to processes of plant tissue culture discussed above, proline is known to accumulate in plant species as a result of environmental stress. Though much is currently known with respect to proline metabolism, certain aspects of its biological functions are not yet clear (Szabados and Savoure, 2009). Proline accumulation is a physiological response in plants, resulting from a wide range of abiotic and biotic stresses (Verbruggen and Hermans, 2008). For example, several plants can accumulate free proline owing to the burden of environmental stresses such as water stress, high salt concentrations, and low temperature (Hare, et al. 1998). These results in proline acting to regulate osmotic changes (Handa, et al. 1986).

Consequently, the accumulation of these compatible solutes (osmoprotectant) e.g. proline, soluble sugar and glycinebetaine, permit turgor stabilization and/or maintenance of proteins and membranes against the destabilizing effects of abiotic stresses; salinity, temperature and drought extremes, all of which leads to reduction of cellular water. Soluble sugar and proline accumulation in plant cell under drought or salt stress is an already established response. These osmoprotectant compounds play a critically important role in osmotolerance and osmoregulation (Gadallah, 1999; Rajasekaran, et al. 1997). It therefore follows that accumulation of solutes like proline, are obvious factors that assistance the plant systems to adaptation under saline conditions (Bohnert, et al. 1995 ; Garcia, et al. 1997).

Furthermore, improvement of salt or drought tolerance of crop plants by engineering proline metabolism is an existing possibility and needs to be explored more extensively. The reality that proline can act as an indicator molecule and control defense pathways, organize metabolic and developmental processes, makes it more favourable for plant improvement. Moreover studies needed to find out the possibility of engineering flowering time or to progress defenses against some pathogens through targeted engineering of proline metabolism (Szabados and Savoure, 2009).

This review summarizes the achievements of applications of plant tissue culture technology and the importance of amino acid proline during the period of developmental abiotic stress.

2.Techniques for Plant Tissue Culture

This section discusses the techniques of plant tissue culture. As already defined above, plant tissue culture is the culturing of plant tissues or cells in an artificial culture medium under laboratory controlled sterile conditions with the resultant production of millions of identical plants. Presently, it has commercial applications as well as value in requisite research into cell biology, biochemistry and genetics (Khatri and Gandhi, 2011). Plant tissue culture has also emerged as an advanced tool and forms the basis of plant biotechnology. The existence of improved varieties of planting materials are a basic need for growers aimed at increasing productivity. (Chebet et al., 2003).

In particular, plant tissue culture is a technique are used for in vitro regrowth of plants. The technique is based on the maintenance of plant cells under sterile conditions on a defined nutrient medium. The culture can be maintained as a mass of undifferentiated cells for a long period of time or regenerated into an entire plant. Plant tissue culture techniques are also crucial to the emergence of new areas of applied plant science, including plant biotechnology and agriculture (Pande and Gupta, 2013).Presently, modern plant tissue culture techniques are carried out under sterile conditions by using filtered air. It is well known that live plant materials from the soil or aquatic ecosystems are naturally contaminated on their outer and inner surfaces by indigenous microorganisms. Therefore, surface sterilization of initial materials (explants) in chemical solutions such as alcohol or bleach is required (Mineo, 1990).

Studies by (Georgiev et al., 2009) have indicate that different techniques in plant tissue culture may have some advantages over traditional methods of plant propagation which include:

1. Regrowth of entire plants from plant cells that have been altered genetically.
2. The production of unaltered copies of plants that generates good flowers, fruits, or having other notable qualities, to output mature plants and multiples of plants in the lack of seeds or necessary pollinators for seed germination.
3. Production of plants from seeds that have lower viability, i.e. nepenthes and orchids. Another production of haploid plants, hybrid productions for incompatible species, applications also include germplasm maintenance, etc.

Further to the above mentioned advantages, the use of tissue culture techniques or cells are good application for salinity studies; they help to resolve the halophytic plants' salt tolerance mechanism at the unorganized tissue level or cellular, and may also supply information on the possibility for growth responses, biochemical and physiological to salt stress at various levels of tissue organization. Another advantage of in vitro studies is that it allows for faster responses, shorter growth period and controlled environment as compared to field studies (Zhang et al. 2004; Pe´rez-Tornero et al. 2009).

2.1 Applications Plant Tissue Culture

This section review the various applications of plant tissue culture. Since the emergence of plant cells and tissue culture as a field of study within plant biology, several investigators have attempted to using plant cell biosynthetic capabilities to obtain useful products and for studying their metabolism (Misawa, 1994; Verpoorte et al., 2002).Presently, plant tissue culture have lineal commercial applications as well as value in requisite research into cell biology, genetics and biochemistry. The techniques involves culturing of embryos, anthers, ovules and cells, protoplast isolation and fusion, cell chosen, bud and merited culture on experimental to industrial levels. (Georgiev et al., 2009).

Sharma and Agrawal, (2012) reported the emergence of *in vitro* tissue culture technique has presented a new process to the morphogenetic investigations. The option of insect, disease, or stress resistant plants using tissue culture techniques have become one of the most intensively studied zone of plant tissue culture in recent times (Jain, 2001).
Studies (Bhat et al., 2013) have notes that different techniques in plant tissue culture may display specific advantages over traditional methods of propagation and these are summarized below:

1. Fast production of mature plants.
2. The production of plants in sterile containers that let them to be moved with lower chances of pathogens, transmitting diseases and pests.
3. To clean specific plants with other infections and viral, and to rapidly multiply these plants such cleaned stock for agriculture.
4. The production of multiples of plants in the non attendance of seeds or natural pollinators.
5. The production of unaltered copies of plants that production perfect flowers, fruits, or have other notable qualities.
6. The regeneration of entire plants from plant cells that have been altered genetically.

2.3. Explant Source

Plant tissue cultures are start from small pieces, known as explants, obtained from any part of a plant. In practice, each parts of a plant have been successfully used as sources of explants. This is achieved by removing the Uexplant" by surgical means, surface sterilized and placed on a nutrient medium to start the parent culture, that is multiplied continuously by subculture. The subsequent plant parts are widely used in commercial micropropagation (Ahloowalia et al., 2004).

Tissue cultures are onset from pieces of entire plants. The part of the plant i.e. the stock plant or parent plant from which the explants are obtained, varies according to the kind of culture to be start, the purpose of the suggest culture while the plant species to be used (George, 2008).

Since they are started from small explants and should be grown on a nutrient media that are as will appropriate for the growth of microorganisms, the plant tissue cultures are normally established and maintained in aseptic circumstance. It is worth noting that most microbes, especially fungi and bacteria, are hostile to plant materials growing in vitro. As a result, efforts should be made to ensure that explants are free from microbial contaminants when they are initially placed on a nutrient medium. This includes growing stock plants in tracks that will reduce infection, treating the plant materials with disinfects or chemicals that can eliminate superficial microbes, sterilizing the tools used for dissection and the containers and media in which cultures are grown (Cassells and Doyle, 2005).

The following many of the plant parts are using in commercial micropropagation (Ahloowalia et al., 2004).

1. Bud culture and floral meristem: although such explants are not commonly used in commercial propagation, unless buds and floral meristems can generate complete plants.
2. Axillary bud or nodal culture: includes of a piece of stem without a portion of the shoot or together with axillary bud culture with. When however the axillary bud is taken, it is specific as "axillary bud" culture.
3. Meristem-tip and Shoot-tip culture: Shoots evolve from a small group of cells recognize as shoot apical meristem.
4. Different sources of explants, e.g. in some plants, immature zygotic embryos, leaf discs, small pieces of stems, intercalary meristems from nodes, and nucellus have as well been used as explants to initiate cultures.
5. Cell suspension and callus cultures: different parts of plant such as ovules, intercalary meristems, stem-pieces, microspores microspores, anthers, pollen, immature embryos and leaf discs have been cultured to induce callus. A callus is a mass of regulates cells, which in many cases, following transfer to suitable medium, can give rise to somatic embryos and shoot-buds, which in turn forms complete plants.

3. Amino Acid Proline

Amino acid proline is the most widespread compatible osmolyte in plants. Proline structure pathway in plants, that takes place in cytoplasm, of glutamate through glutamyl-γ-semialdehyde and γ-glutamyl phosphate. The enzyme pyrroline-5-carboxylate synthetase (P5CS) in plants catalyzes this response in two stages. The glutamyl-γ-semialdehyde is naturally cyclised to pyrroline-5-carboxylate, which is transform to proline through pyrroline-5-carboxylate reductase (Taylor, 1996).Proline accumulation in plants, has been reported to occur following salt, , heavy metal, pathogen infection, anaerobiosis, nutrient deficiency, drought, low temperature, high temperature, UV irradiation and atmospheric pollution (Siripornadulsil et al. 2002).Therefore, the capacity for proline hyper accumulation is associated with the extremophile character of specific plant species and is potential that it participate to their stress tolerance. While, it is not an absolute demand for adaptation to extreme environmental conditions (Szabados and Savoure, 2009).

3.1 Proline Accumulation

The accumulation of proline in plants has been reported to occur after abiotic striess (Siripornadulsil et al. 2002). Further, proline occurs widely in higher plants and in addition to accumulating in greater amounts than other amino acids in salt affected plants (Ashraf 1994; Abraham et al. 2003). The proline accumulation is an ordinary physiological response to different stresses however it is also part of the evolution program in generative tissues like pollen. Studies have shown that

transgenic approaches have assured the beneficial effect of proline overproduction through different stress (Verbruggen and Hermans, 2008). As referred by Sharma and Dubey, (2005) these compatible osmo protectants or solutes can accumulate to high levels without interrupting intracellular biochemistry. The increase levels of the osmolytes accumulated in plant cells correlate with increased stress tolerance through scavenging of protecting enzymes and free radicals.

Salt stress may as will induce changes in the composition of especially of proteins, free amino acids (FAA) and N-containing compounds. The accumulation of proline has been extensively, used as an indicator of stress tolerance or of salt stress (Ashraf and Foolad, 2007).This results in an increase of free amino acids (FAA) which is readily regarded as an enhance of tolerance to salinity.

3.2 The Importance of Proline During Development and Stress

Proline contributes to the stability of sub-cellular structures (e.g. proteins and membranes) and also scavenging free radicals respectively (Apel and Hirt, 2004). In addition to buffering cellular redox potential under difference stress (Ashraf and Foolad, 2007). Proline accumulation with raised concentrations of NaCl, might be part of the strategies adapted by plants to survive under stress conditions (Khorami, et al., 2011).Accumulation of proline is one of the most readily reported mechanisms induced in plants through salt stress, and it is predominantly considered to be involved in stress resistance. Studies have also found that plant adaptations to salinity contain isolate of salt ions in vacuoles to maintain ionic homeostasis in the cells, the rise proline accumulation was in 50 mM concentration of NaCl and the lowest was in the no stressed situation (Khorami et al., 2011).Further, proline accumulation is a combined characteristic in many monocotyledons at saline conditions. Important proline regulates the accumulation of available N, which is osmotically active (Ashraf 1994).

Other studies have also proposed functions relating to proline accumulation, including stabilization of macromolecules, a exporter of nitrogen and carbon to be used after plants are relieved from water stress (Samaras, et al. 1995 ; Smirnoff and Stewart 1985). Previous investigators have found that proline accumulates under salt stress in both root and leaf tissues (Aziz et al., 1999)and is supposed to protection against the osmotic potential generated through salt (Watanabe et al., 2000, Chen et al., 2007). Yancey, et al. (1982) found the adaptation to stresses is linked together with metabolic changes that lead to the accumulation of several organic compounds such as proline, sugars, betaines and polyols in plants.

The compatible solutes are classified into two groups: (1) nitrogen-containing complexes for example proline and other amino acids, quaternary ammonium compounds and polyamines. (2) hydroxyl complexes, for example oligosaccharides, sucrose and polyhydric alcohols (McCue and Hanson 1990). An alteration in proline composition has been correlated with its capacity to tolerate and adapt to saline conditions. For example, proline accumulation in the calli exposed to concentration of NaCl medium in the absence of 2,4-dichlorophenoxyacetic acid (2,4-D) was significantly higher than that of the calli stressed at the same salt containing Auxin 2,4-D. The major proline accumulation in the 20 Gy irradiated calli cultured on NaCl medium relative to the non-irradiated calli exposed to the particular salt concentration was partner with higher growth improved (Patade and Suprasanna, 2009).Although proline is exist in halophytes from a large range of families, however, Tipirdamaz et al. (2006) indicate that 'species that acted as glycinebetaine accumulators include modicum proline and *vice versa*'. In *Melaleuca* spp., sundry proline analogues and methylated proline components are the main organic solutes (Naidu et al., 2000).

Studies by Maggio et al. (2002) suggest that proline may act as a signaling and regulatory molecule, capable to invigorate multiple responses effect that is factors of the adaptation process. Proline concentration in numerous salt tolerant plants has been found to be greater than those in salt sensitive ones. Other roles of proline include stabilizing subcellular structures, scavenging free radicals and buffering cellular redox potential in stresses. However salinity stress responsive genes, these genes contractor contain proline responsive elements (ACTCAT, PRE), are as well known to be induced during proline (Chinnusamy et al., 2005).

4. CONCLUSION

The developed of plant tissue culture as a separate branch of science following the discovery of the potentials of each tissue or cell of plant to grow into a separate organism. That techniques at tissue culture has help to opened up new opportunities in improve a new varieties and hybrids, as well as solving many difficult problems traditionally difficult to tackle by other known methods. For instance, in agricultural research. Basically, plant tissue culture involves separation of tissues or cells of plants and growing usually them in aseptic conditions, but also providing suitable nutrients medium under controlled light and temperature. These can be achieved under laboratory conditions. The excised explants (parts of tissues) grow into an entire plant when they are supported with micronutrients, vitamins, sucrose as well as suitable hormones at appropriate culture stages. Proline accumulation is physiological response in a lot of plants in response to wide range of abiotic and biotic stresses. The fact that proline can action as a signaling molecule and also effects defense pathways, regulate complex metabolic also development processes display additional way for plant improvement.

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