eJBio

Electronic Journal of Biology, 2016, Vol.12(4): 422-425

In Vitro Regenerated Plants Response to Seismomorphogenic Stimuli

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Citation: Sarmast MK. *In Vitro* Regenerated Plants Response to Seismomorphogenic Stimuli. Electronic J Biol, 12:4

Received: June 13, 2016; **Accepted:** August 24, 2016; **Published:** August 31, 2016

Short Communication

Abstract

Plant species as sessile organisms must cope with diverse external mechanical stimuli throughout their life cycle which may profoundly influence different facets of their growth and development. These mechanical stimuli, however, may further benefit these plants to orient themselves with other types of biotic and abiotic stresses, and this is commonly termed cross-adaptation. Acclimation is a final step of plant micro-propagation procedure. The application of mechanical stress is a common practice for vegetable crop seedlings before being transplanted into the field. This technique can be potentially applied as a pre-acclimation or acclimation treatment while plantlets undergo the last phase of micro-propagation process. Plantlets subjected to a seismic stress are more likely to cope better with conditions of the external environment. The possible mechanism whereby plants acquire this short and long term adaptation can contribute to the cross-talk of several complicated components such as ROS burst, calcium binding proteins (calmodulin), hormones, MAPK, HSPs, proline and RSRE. Premeditated seismic stress just before transplanting of the *in vitro* regenerated plants may unravel new facet(s) of *in vitro* acclimatization, but its applicability in different plant species and the possible mechanism behind this adaptation deserve to be investigated more extensively in the future.

Keywords: Tissue culture; Seismomorphogenesis; General stress response; Acclimation.

1. Introduction

Millions of years of evolution have adapted plants not to relocate when they face unprecedented environmental conditions. Instead, they have evolved many very complicated functional strategies to confront prevailing environment conditions throughout their life cycle. Probably, the first report aiming at the explanation of mechanical stimuli effects on plant growth and development belongs to Charles Darwin [1]. In nature, the plant firstly needs to sense different external stimuli whereupon there is a rapid response which mostly involves transcriptase modification and then there would be a late stress response. Plant growth responses to tactile or contact stimuli have been termed thigmomorphogenesis. In this form, the subject (here the plant) always has physical contact with objects surrounding it, by ways such as rubbing, impedance, perturbing, bending or flexing, while responses to shaking or vibrational stimuli have been termed seismomorphogenesis in which plants are indirectly influenced by the objects by means of shaking and vibration. To a great extent, the seismo/thigmomorphogenesis is known to affect different aspects of ornamental plants, vegetables and model plants with regard to their growth and development. However, there are very few, if any, reports that underpin the effects of mechanical stress on tissue culture-regenerated plants so as to improve their acclimation efficiency after transplanting. Even though many key players of mechanical stimuli have been unraveled, it is still on boundaries of uncertainty to claim that the same elements are also involved in the realm of *in vitro* culture conditions.

2. Plant Tissue Culture and Acclimation Strategies

Nowadays, micro-propagation has profoundly grown to develop, leading to the production of an oodles number of true–to–type and pathogen free plants in fair amounts of time. *In vitro* plant production normally has three to four steps including the establishment of explants, multiplication, rooting and acclimatization. Plant tissue culture and biotechnology techniques have gradually outweighed classical breeding approaches. The acclimatization of tissue culture-derived plantlets is an *in vitro* practice that has not been greatly acknowledged as yet. These *in vitro*-derived plants do not seem to have a predisposition to cope with unfavorable outside conditions. Explants that are deliberately grown in heterotrophic high relative humidity regimes would exhibit superior growth and development. Unprepared plantlets upon their transplanting to *ex vitro* conditions would face great casualty. Therefore, in order to alleviate these stresses, plants firstly need to acclimatize gradually and then completely move to the greenhouse or field. The mortality percentage of plantlets is even worse for those that have grown in liquid culture media. Plantlets grown under *in vitro* conditions are often constrained by low space, low irradiance, high RH, high concentrations of Plant Growth Regulators (PGRs), high carbohydrate levels, and improper gas exchange. This innocuous condition would help the explants grow faster and basically will not help the resultant plantlets to be fully autotrophic. *In vitro* growing plantlets usually contain malformed and unresponsive stomata and poor epicuticular waxes. Vividly, this physiological disorder cannot stop plants from evapotranspiration upon transplanting. Several recommended acclimatization protocols have been developed so as to increase plantlets durability and performance soon after transplanting. These include the application of several growth retardants such as paclobutrazol and ancymidol. Also, gas exchange and CO_2 enrichment, sugar-low and sugar free media are employed to make plantlets autotrophic. Plantlets may further be prepared against harsh outside environments by reduced humidity, root induction prior to transplanting, reduction in the concentration of media salts or by using media with lower levels of nitrogen, in some cases increased levels of agar, elevated irradiance, the use of effective innocuous foliar anti-transpirants and removing the baby jars caps. Herein, it is hypothesized that seismomormophogenetic, but not thigmomorphogenetic treatments, can be an alternative option for acclimatization of *in vitro* regenerated plants. In case of tissue culture-regenerated plants, it would appear less successful to start this treatment outside baby jars for two reasons. First, plantlets are too sensitive to be able to endure thigmomorphogenetic treatments, and therefore may be hurt. Second, *in vitro* derived plantlets do not have complete control over their evapotranspiration machinery system, thereby losing their water potential. This could lead to the plant's demise upon being transplanted, before being fully acclimatized. Therefore, one logical option is to use the seismic approach at the end of the micro-propagation process while plantlets are still growing inside the baby jars. These techniques can prove to prepare explants against stresses of the outside environment. Expediency of mechanical stimulation as a nifty tool for acclimatization of tissue culture regenerated plantlets is reported successfully herein. The process involves shaking the *in vitro* growing *Sansevieria trifasciata* L. plantlets twice daily at 6:00 a.m. and 9:00 p.m. for 2, 4, 8 and 16 min at 250 rpm for 14 days. Just after the end of the treatments, leaf length decreased but proline content of leaf and root length increased (by a two-fold increase in root length), suggesting a short-term response in order to maintain cellular homeostasis against the unfavorable condition. More specifically, sixteen-minutes of shaking at 250 rpm decreased leaf length in the control from 5.11 cm to 3.90 cm which was not significantly different at $P \leq 0.05$. Treatments of more than 8 min shaking at 250 rpm increased proline content in comparison with control plants. Six months after starting the experiment, it was astoundingly observed that explants which had received 16 min of shaking treatment had longer leaf length ($P \leq 0.05$) and greater leaf area. Furthermore, their photosynthesis rate increased as a result, compared to control plantlets. It has been confirmed in different plant species that plants which had received mechanical stimulation could also endure other forms of stress [2]. According to the above statement, there is a high chance for plantlets to survive after transplanting when they received mechanical stress treatment during *in vitro* growing condition. It is hypothesized hereby that cross-adaptation most probably is involved in regulating these responses. The plantlets that have received mechanical stimuli would gradually adapt themselves against stresses in later stages. These cross-adaptation strategies have also been reported concerning tobacco cell culture [2]. Mechanical stress as a cost-effective and non-chemical approach could potentially improve plants quality and reduce mortality percentage.

3. Morphophysiological Changes

Plants underwent great morphological changes in order to adapt themselves to external abiotic stimuli. Inhibition of plant growth is the first visible result of mechanical stress treatments. There is a correlation between peroxidase activity and plant lignification which, after mechanical stress, will culminate in the inhibition of elongation. Cuticle thickness and stomata number were also affected soon after mechanical stimuli. It has been shown that lignin percentage increased after touch and water spray treatments. Reduced deposits of epicuticular waxes, the incapability of the plantlet stomata to close shortly after removal from culture, and deficient root system are the pivotal causes for water loss from leaf, desiccation, and poor survival after transplanting [3].

3.1. PGRs

Several reports collectively hypothesized that inhibition of plant stem elongation is because of disruption in polar auxin transfer. In addition, in response to shaking treatment, gibberellic acid-like hormones decrease after seismic treatment, but in contrast the ABA increased. The production of ethylene in the small space of culture vessels cannot be excluded. The external application of ethylene could mimic the thigmomorphogenetic response. The ethylene role in radial expansion aspects of thigmomorphogenesis has been previously reported [4].

The role of cytokinin during mechanical stress-mediated response is not fully understood. Jasmonic acid (JA) and its precursor 12-Oxo-10,15-phytodienoic acid (12-OPDA) are also to some extent involved in thigmomorphogenesis-mediated response. These ideas originate from research on wild cucumber, *P. vulgaris*, *Medicago trancatula* and Arabidopsis [5]. The cross-talk among hormonal signaling needs further attention in plants subjected to mechanical stresses. But more importantly, the aim of research is to decipher the key role(s) of the aforementioned hormones and how they play such roles in mechanical stress-affected *in vitro* plants.

3.2. Proline

Proline accumulation is another physiological response to some of the studied mechanical stresses. Proline has been shown to function as a molecular chaperone that enables protection of protein integrity and enhances the activity of different enzymes. Along with its signaling activity, proline acts as a singlet oxygen quencher and has shown ROS scavenging activity as well [6]. It has also been stated that mechanical stress influenced intracellular Ca^{2+} levels in algae [7]. The type of mechanical stress which is induced by the increase in speed of rotary shaker during tobacco suspension culture resulted in an improved survival percentage when plantlets were exposed to chilling stress, and there was also a regrowth ability of tobacco suspension cells during normal culture conditions after chilling stress. Intriguingly, proline may have become a biomarker for mechanical stresses *in vitro* [2,3]. Heat, salt and heavy metal tolerance acquisition has been previously shown in tobacco cells subjected to mechanical stress *in vitro* [8].

3.3. Reactive oxygen species and Ca^{2+}

ROS (reactive oxygen species) is another signaling molecule during mechanical stress through which Ca^{2+} channels conduiting intracellular Ca^{2+} during the course of mechanical stress [9,10]. ROS and Ca^{2+} are two key elements that rapidly induce upon mechanical stress, in fact augmented cytosolic calcium partly can transfer through calmodulin binding protein, or calcineurin B-like proteins along with CIPKs (calcineurin interacting protein kinases) and CDPKs (calcium-dependent protein kinases) into the complicated downstream molecule like MAPK (Mitogen-Activated Protein Kinase), ROS and etc., to activate general stress response genes. The release of many antioxidant enzyme during suspension cultures of *Taxus cuspidate* subjected to shear stress may be due to their role in scavenging ROS [11].

3.4. Nitric oxide

Nitric oxide (NO) - a colorless gas - accumulation was observed in tobacco suspension-cultured cells subjected to mechanical stress. It has been hypothesized that ROS, NO and Ca^{2+} together modulating mechanical stress response in plants [5]. Different NO role during biotic and abiotic stress as a signaling molecule to great extend reviewd [12]. However it's key regulatory role in mecanical stress-induced reaction needs to be determined.

3.5. Rapid stress response element

The promoter segments of some general stress genes upregulated by wounding stress overrepresented some elements so called Rapid Stress Response Elements (RSRE). RSRE further manifested the transcriptional modules role during general stress response. It has been confirmed that these cis-regulatory elements are a target site for Calmodulin-binding Transcriptional Activator (CAMTA) family. It is necessary to distinguish the long term effects, if any, of mechanical stress on CAMTA - a rapidly and transiently wound-responsive molecule.

3.6. Heat shock proteins

Heat Shock Proteins (HSPs) are chaperon molecules inside the cell. It has also been shown that different forms of Heat Shock Protein (HSP) can be produced during shear stress in suspension cultures of *Taxus cuspidate.* Despite the fact that HSPs are presented in different kinds of stress conditions, their regulatory role during mechanical stress-mediated response is yet to be determined.

In order to find out which approach serves better the purpose of acclimatization *in vitro,* a comprehensive and comparative study needs to be asserted to increase our understanding of *in vitro*-regenerated plant acclimatization.

4. Molecular Changes

In response to thigmo/seismomorphogenesis, many genes with functions such as calcium sensing, cell wall modifications, and also defense antioxidant-related genes have been affected [5]. Conserved touch–inducible plant genes are called *TCH* and have been discovered by Braam and Davis [13]. *TCH1* encode Ca^{2+} sensors called calmodulin-binding proteins, *CAM2*. Calmodulin-like (CML) proteins such as *CML24* and *CML12* are encoded by *TCH2* and *TCH3* respectively. However only *CAM2=TCH1* was identified by microarray analysis as being touch-inducible. In addition, *TCH4* encodes a Xyloglucan endotransglucosylase/hydrolases (*XTHs*) which are cell wall modifying proteins involved in mechanical stress. They contribute to fundamental processes in the plant cell expansion [4]. Microarray has revealed that a substantial amount of genes are unregulated by touch and are also up-regulated by darkness, partially suggesting the commonality role of the aforementioned genes in different type of stress.

It is clear that other genes involved in mediating biosynthesis and signaling of key elements in mechanical stress such as protein kinase would play substantial roles in this realm [4].

In summary, the lack of specially designed equipment for shaking the *in vitro* plantlets in large scale is a constraint besides justifications regarding the duration of treatment and the speed of shaking. Mechanical stress must be served based on the species, its developmental stages and sensitivity to stimuli. Researchers need to take this into consideration that most plants don't respond equally to the mechanical stress. In addition, the interactions between mechanical stress, temperature and light intensity would be new dimensions to explore. Mechanical stress-mediated acclimatization of *in vitro* regenerated plantlets is probably worth to be more investigated in future research since it serves as a non-chemical and cost-effective approach. Its expediency on potentially improving plants' quality and reducing mortality percentage of plantlets provides an open field for future research.

5. Acknowledgement

Mohsen Hamedpour-Darabi is thanked for editing the language of the paper.

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