In Vitro Breeding of Heavy Metal-Resistant Plants: A Review

Seyedardalan Ashrafzadeh and David M.W. Leung*

School of Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch 8140, New Zealand

*Corresponding author: david.leung@canterbury.ac.nz

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Abstract. Plant biotechnology using in-vitro cell and tissue culture is a practical plant breeding tool in developing plants resistant to different abiotic stresses such as cold stress and elevated soil salinity. In this study, the focus is on the in vitro breeding method applied for development of plants resistant to heavy metal (HM) stress. It consists of the following three successive stages: (i) initiation of callus cells, some of which are somaclonal variants with new traits, (ii) exposure of the calli to HMs as selective agents during proliferation for selection of somaclonal variants with enhanced HM-resistance, and (iii) selection of the desirable resistant variants following plant regeneration in the presence of HMs. The whole procedure is more efficient and cost-effective than the conventional breeding methods. Moreover, the plants developed through this approach are not regarded as genetically modified organisms (GMOs), and therefore, did not pose negative public acceptance issues unlike GM plants. However, despite the numerous advantages of this in-vitro breeding approach, it has been employed in a few plant breeding studies to generate HM-resistant plants. The present study outlined the fundamental principles of in vitro breeding and the progress made so far towards development of HM-resistant plants based on this approach.

Additional key words: abiotic stress, heavy metal tolerance, somaclonal variation, tissue culture

Introduction

Soil contamination with heavy metals (HMs) has become an important public health and food production concern in the past few decades (Sharma and Agrawal, 2005). This problem occurs due to increased human activities such as mining, fertilizer application, and industrial development (McLaughlin et al., 1999). Heavy metals from anthropogenic activities are added to the natural levels released from bedrocks and are persistent in the environment as their potential ecological and health threats cannot be simply removed with the passage of time by microbial degradation. There are many proposed strategies for remediation of soil heavy metal pollution and the use of plants (phytoremediation or phytotechnologies) is of particular interest. Conceivably, the various ways in which plants could be used in the management of heavy metal pollution problems may include: (i) phytoextraction (to reduce soil HM levels), (ii) phytostabilization (to reduce soil erosion, leaching and runoff of HMs by in situ immobilization with the help of plant root chemistry and thereby minimizing bioavailability of heavy metals), and (iii) phytovolatilization (uptake by plant roots from soil and then converted by plant cells to volatile forms that can be released into the atmosphere (Pilon-Smits, 2005).

There are many challenges for phytoremediation of soil heavy metal contamination. A basic pre-requisite for phyto-management of soil heavy metal problems is that metal-resistant plants must be used following their identification or development via traditional plant breeding or biotechnology. There is no previous report of in vitro breeding of HM-resistant plants based on somaclonal variation. Therefore, here the fundamental principles and basic methodology of the in vitro breeding approach as applied to development of HM-resistant plants were first outlined. In addition, the few studies showing HM-resistant plants were discussed including characterization of the callus cultures and HM-resistant plants obtained. It is hoped that with a basic understanding of the tissue culture-based development of HM-resistant plants and a discussion of the existing obstacles affecting the efficiency of this in vitro breeding method would stimulate more research into this technology.

In Vitro Plant Breeding for Improved Heavy Metal Resistance

Plants use different strategies to respond to the presence of elevated bioavailable HMs in the environment. The HMs
in soils can be taken up by plant roots and then a portion of the absorbed HMs might be distributed among different plant organs (Mari and Lebrun, 2006). In some plants, even limitation of HM translocation from the roots to the above-ground organs would be of survival value so that photosynthesis could be protected to sustain plant growth (Nocito et al., 2011). Plants may also have evolved HM avoidance or exclusion mechanism so that HM uptake from soils would be reduced or limited (Ahmad et al., 2007; Liu et al., 2009; Seregin et al., 2014; Wei et al., 2005). The HMs are known to induce oxidative stress in plant cells disrupting plant metabolism and growth (Bhaduri and Fulekar, 2012). Therefore, another important strategy is activation of antioxidative enzymes counteracting reactive oxygen species (ROS) produced by HM stress (Hall, 2002; Schützendübel and Polle, 2002) and thereby minimizing the adverse impacts of oxidative stress on the plant cells (Ernst, 2006). Obviously, plants which cannot develop an efficient defense mechanism, can hardly survive in HM-polluted soils. Research approaches that can result in enhanced HM resistance in plants are of particular interest.

**Traditional Breeding vs. Tissue Culture-Based Breeding**

Although it is well-documented that natural variation in HM uptake and resistance occurs among different plant species and genotypes (Grant et al., 2008), it is not always broad enough to permit selection of the tolerant variants. To improve plant HM-resistance, application of conventional breeding methods can be an alternative but due to the lengthy procedures and high costs required, plant breeders seldom give the HM resistance trait as the priority unlike the more traditional targets such as drought, salinity or biotic stress that affect yield.

In vitro breeding or use of plant tissue culture techniques can induce variation via somaclonal variation phenomenon. Therefore, the variant plants obtained are not regarded as GMOs (genetically modified organisms via novel gene biotechnology manipulations) as no in vitro-manipulated or recombinant DNA is transferred to generate the variants. Afterwards, variants of interest (with high HM-resistance) can be easily selected through this approach under highly controlled conditions. Unlike traditional plant breeding, there will be a minimal requirement of space and time for in vitro breeding. Indeed, in a recent review on in vitro breeding for abiotic and biotic stresses in plants there are many studies on generation of variants with improved drought, salinity and disease resistance but by comparison only a few HM-resistant plant variants were obtained in this way (Rai et al., 2011).

**HMs as Selecting Agents in Plant Tissue Culture Media**

In vitro breeding basically starts with explants removed from mother plants grown under in vitro conditions (Fig. 1). Fully-developed plant structures including the leaves and shoots may be used as the initial explants which can be cultured on a common basal plant tissue culture medium such as Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) supplemented with different combinations and concentrations of plant growth regulators (PGRs). The two principal PGRs often used are auxins and cytokinins which in optimized concentrations can trigger totipotent cells to form undifferentiated parenchyma cells called callus cells (calli) (Gaspar et al., 1996). Callus initiation starts from edges or wounded parts of explant tissues and then can gradually cover whole of the explant surfaces. The appropriate culture conditions such as optimized temperature and lighting are the other requirements provided in a control chamber or growth room. The time and frequency of callus induction can vary with plant species and genotype (Sharma and Agrawal, 2005).

Callus cells are mostly proliferated on an agar-solidified medium containing the same combinations and concentrations of phytohormones as used in the initiation medium. Otherwise, cell suspension culture in liquid medium can be developed but has been used less often in the in vitro breeding literature presumably because establishment and maintenance of cell suspension cultures would require extra efforts and resources (Table 1). At the early stage of callus initiation and proliferation, the chance of somaclonal variation occurrence is very high (Wang and Wang, 2012). It may be advantageous to add a HM of interest in the culture medium at this stage to help to select for any HM-resistant somaclonal variant cells. The callus proliferation stage can take from a few weeks to a few months (Table 1). Prolonged subcultures with stepwise increases in the concentrations of HMs could also help to capture somaclonal variation occurrence. However, the regenerating ability of calli might be adversely affected with prolonged subculture (Bairu et al., 2011; Kaepppler et al., 2000). As the callus cells are not homogeneous, different exposure periods and levels may trigger different responses from the different constituent cell populations in the proliferating callus culture. Some phytohormonal treatments may trigger callus cells to undergo embryogenesis instead of proliferation and the embryos formed may also exhibit HM resistance (Von Arnold et al., 2002).

Calli should be exposed to the stress factors or selecting agents (HMs) during proliferation (subculture) or even from earlier (the callus induction stage) to select for the desirable trait (HM-resistance). Exposure of callus to a sub-lethal concentration of HM has been applied more than stepwise increases in HMs in the previous studies (Table 1). This is...