Environmental and Experimental Botany 71 (2011) 89–98

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Developing stress tolerant plants through *in vitro* selection—An overview of the recent progress

Manoj K. Rai[a,*], Rajwant K. Kalia[a,b], Rohtas Singh[a], Manu P. Gangola[a,c], A.K. Dhawan[a]

[a] Centre for Plant Biotechnology, CCSHAU New Campus, Hisar 125004, Haryana, India
[b] Botany and plant sciences, 2150 Batchelor Hall, University of California, Riverside, CA 92521, USA
[c] Department of Plant Sciences, 51 Campus Drive, College of Agriculture and Bioresources, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 5A8

ARTICLE INFO

Article history:
Received 20 December 2009
Received in revised form 19 October 2010
Accepted 29 October 2010

Keywords:
Abiotic stress
Antioxidative enzymes
Disease resistance
Osmotic adjustment
Reactive oxygen species

ABSTRACT

Biotic and abiotic stresses impose a major threat to agriculture. Therefore, the efforts to develop stress-tolerant plants are of immense importance to increase crop productivity. 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 both the biotic and the abiotic stresses can be acquired by applying the selecting agents such as NaCl (for salt tolerance), PEG or mannitol (for drought tolerance) and pathogen culture filtrate, phytotoxin or pathogen itself (for disease resistance) in the culture media. Only the explants capable of sustaining such environments survive in the long run and are selected. *In vitro* selection is based on the induction of genetic variation among cells, tissues and/or 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. This review focuses on the progress made towards the development of stress-tolerant lines through tissue culture based *in vitro* selection. Plants have evolved many biochemical and molecular mechanisms to survive under stress conditions. The mechanisms of ROS (reaction oxygen species) generation and removal in plants under biotic and abiotic stress conditions have also been reviewed.

1. Introduction

The production and productivity of several crops continues to be adversely affected due to various biotic and abiotic stresses. Damages caused by these stresses are responsible for enormous economic losses worldwide. Traditional breeding technologies and proper management strategies continue to play a vital role in crop improvement. The conventional breeding programmes are being employed to integrate favorable genes of interest from inter crossing genera and species into the crops to induce stress tolerance. However, conventional breeding methods have little success and have failed to provide desirable results (Purohit et al., 1998). Therefore, we need to deploy the biotechnological tools for addressing the critical problems of crop improvement for sustainable agriculture.

Genetic engineering for developing stress tolerant plants, based on introgression of genes that are known to be involved in stress response and putative tolerance, might prove to be a faster track towards improving crop varieties. Genetic transformation is now a widely used procedure for introducing genes from distant genepools into many plant species for the development of stress tolerant plants and considerable efforts have been made to produce stress-tolerant plants using this technique (Borsani et al., 2003; Yamaguchi and Blumwald, 2005). However, the major limiting factors in extension of this technique to several stresses are the silencing of transgene, consequent reduction of gene expression and low transformation frequency (Mondal et al., 1997). Tissue culture technique has emerged as a feasible and cost-effective alternative tool for developing stress-tolerant plants in recent years. This technique can operate under controlled conditions with limited space and time (Sakhanokho and Kelley, 2009), and has the potential for selection of stress-tolerant variants using a low cost laboratory set up.

In this review, the progress made towards the development of stress-tolerant plants through tissue culture based *in vitro* selection has been discussed. The physiological and biochemical changes leading to improved stress-tolerance under *in vitro* conditions are also reviewed.

2. *In vitro* selection pressure technique

In vitro culture of plant cells, tissues or organs on a medium containing selective agents offers the opportunity to select and

* Corresponding author. Present address: Biotechnology Centre, Department of Botany, Jai Narain Vyas University, Jodhpur 342033, Rajasthan, India.
E-mail address: mkraibhu@gmail.com (M.K. Rai).

0098-8472/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.envexpbot.2010.10.021

regenerate plants with desirable characteristics. The technique has also been effectively utilized to induce tolerance which includes the use of some selective agents that permit the preferential survival and growth of desired phenotypes (Purohit et al., 1998). The selecting agents usually employed for *in vitro* selection include NaCl (for salt-tolerance), PEG or mannitol (for drought-tolerance), specific fungal culture filtrate (FCF) or phytotoxin such as fusaric acid or the pathogen itself (for disease-resistance). The explants are exposed to a broad range of these selective agents added to the culture medium. Only the explants capable of sustaining such environments survive in the long run and are selected. Two types of selection methods has been suggested: (a) stepwise long-term treatment, in which cultures are exposed to stress with gradual increase in concentrations of selecting agent and (b) shock treatment, in which cultures are directly subjected to a shock of high concentration and only those which would tolerate that level will survive (Purohit et al., 1998). These methods are based on the induction of genetic variation among cells, tissues and/or organs in cultured and regenerated plants (Mohamed et al., 2000). The tissue culture induces variation in regenerated plants, called somaclonal variation (Larkin and Scowcroft, 1981), can result in a range of genetically stable variations, useful in crop improvement. *In vitro* selection can considerably shorten the time for the selection of desirable traits under selection pressure with minimal environmental interaction, and can complement field selection (Jain, 2001). Despite many advantages, development of stress tolerant plants through *in vitro* selection has some limitations like loss of regeneration ability during selection, lack of correlation between the mechanisms of tolerance operating in cultured cell, tissue or organ and those of the whole plants, and phenomenon of epigenetic adaptation (Tal, 1994). During *in vitro* selection, non-tolerant cells sometimes can undergo an epigenetic adaptation i.e. stable epigenetic alterations that are inherited only through mitosis and not through meiosis, to the specific selective agents thus obscure the selection of rare mutants with true tolerance, i.e. meiotically inherited (Tal, 1994). Numerous studies document that altered DNA methylation is a major cause of epigenetic modifications, which are sometimes observed in tissue cultured experiments (Guo et al., 2007; Li et al., 2007; Gao et al., 2010). Many authors suggested that the problem of epigenetic adaptation during *in vitro* selection can be overcome by the use of short-term or one-step selection that may prevent the development of epigenetically adapted cells (Chandler and Vasil, 1984; McHugen and Swartz, 1984; Dix, 1993; Tal, 1994).

3. Development of abiotic stress-tolerant plants through *in vitro* selection

The productivity of several commercial crops is limited by major abiotic stresses including salinity, drought, water logging, heat, frost and mineral toxicities. Successful application of biotechnology to abiotic constraints being faced by these crops will require knowledge of good biological information regarding the target species as well as the mechanisms underlying resistance/tolerance to these stresses (Dita et al., 2006). Development of abiotic stress tolerant plants specially for salt and drought conditions using *in vitro* selection has been reported in a wide range of plant species including cereals, vegetables, fruits and other commercially important plant species (Tables 1 and 2).

3.1. Development of salt-tolerant plants

Soil salinity existed long before humans and agriculture but the problem has been aggravated by agricultural practices such as irrigation and poor drainage systems. Salinity in agricultural fields is thus a severe constraint to crop growth and productivity in many regions and the situation has become a global concern. It is estimated that 20% of the irrigated land in the world is affected by salinity (Yamaguchi and Blumwald, 2005). Moreover, it has been predicted that increasing salinization in agricultural fields will reduce the land available for cultivation by 30% within the next 25 years, and up to 50% by the year 2050 (Wang et al., 2003).

Excess salt in soil or in solutions interferes with several physiological and biochemical processes, resulting in problems such as ion imbalance, mineral deficiency, osmotic stress, ion toxicity and oxidative stress; these conditions ultimately interact with several cellular components, including DNA, proteins, lipids, and pigments in plants (Zhu, 2002), impeding the growth and development of a vast majority of crops. The protection of crops against salinity-

Table 1
Screening and *in vitro* selection for NaCl-tolerance.

Plant species	References
Brassica oleracea (cauliflower)	Elavumoottil et al. (2003)
Brassica juncea (Indian mustard)	Jain et al. (1990)
Brassica napus (rapeseed)	Rahman et al. (1995)
Chrysanthemum morifolium (chrysanthemum)	Hossain et al. (2007)
Citrus aurantium (sour orange)	Koc et al. (2009)
Citrus limon (lemon)	Piqueras et al. (1996)
Citrus sinensis (shamouti orange)	Ben-Hayyim and Goffer (1989)
Cymbopogon martinii (palma rosa)	Patnaik and Debata (1997a)
Cynodon transvaalensis × *C. dactylon* (bermuda grass)	Lu et al. (2007)
Dendrocalamus strictus (bamboo)	Singh et al. (2003)
Diplachne fusca (kaller grass)	Nanakorn et al. (2003)
Fragaria × *ananassa* (strawberry)	Dziadczyk et al. (2003)
Glycine max (soya bean)	Liu and Staden (2000)
Hordeum vulgare (barley)	Ye et al. (1987)
Helianthus annus (sunflower)	Davenport et al. (2003)
Ipomoea batatas (sweet potato)	He et al. (2009)
Linum usitatissimum (flax)	McHughen (1987)
Lycopersicon esculentum (tomato)	Kripkyy et al. (2001)
Lycopersicon peruvianum (wild tomato)	Hassan and Wilkins (1988)
Medicago sativa (alfalfa)	McCoy (1987), Safarnejad et al. (1996)
Morus sp. (mulberry)	Vijayan et al. (2003)
Nicotiana tabacum (tobacco)	Rout et al. (2008)
Oryza sativa (rice)	Binh and Heszky (1990), Basu et al. (1997), Shankhdhar et al. (2000), Lee et al. (2003)
Saccharum sp. (sugarcane)	Gandonou et al. (2006)
Solanum tuberosum (potato)	Sabbah and Tal (1990), Ochatt et al. (1999), Queiros et al. (2007)
Triticum aestivum (wheat)	Vajrabhaya et al. (1989), Karadimova and Djambova (1993), Barakat and Abdel-Latif (1996), Zair et al. (2003)
Vigna radiata (mungbean)	Hassan et al. (2008)