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SHORT COMMUNICATION

Effect of physical desiccation on plant regeneration efficiency in rice (*Oryza sativa* L.) variety super basmati

Ikram-ul-Haq[a,*], Zhao Chang-Xing[b], Zahid Mukhtar[f], Cheruth Abdul Jaleel[c,*], Mohamed Mahgoub Azooz[d,e]

[a]*Institute of Biotechnology and Genetic Engineering (IBGE), University of Sindh, Jamshoro, Pakistan*
[b]*College of Agronomy and Plant Protection, Qingdao Agricultural University, Qingdao 266109, China*
[c]*Stress Physiology Lab, Department of Botany, Annamalai University, Annamalainagar 608 002, Tamilnadu, India*
[d]*Department of Botany, Faculty of Science, South Valley University, 83523 Qena, Egypt*
[e]*Department of Biology, Faculty of Science, King Faisal University, P.O. Box 380, Al-Hassa 31982, Saudi Arabia*
[f]*National Institute for Biotechnology and Genetic Engineering (IBGE), P.O. Box 577, Faisalabad, Pakistan*

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Physical desiccation;
Plant regeneration efficiency;
Relative callus proliferation rate;
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Summary

This experiment assessed the effect of partial physical desiccation on plant regeneration efficiency in scutellum-derived embryogenic calluses of rice (*Oryza sativa* L.) variety Super basmati. A number of callusing cultures were developed, and efficient callus induction was observed on MS (Murashige and Skoog) basal medium supplemented with 2.0 mg/L 2,4-dichlorophenoxy acetic acid. The calluses were proliferated on the same medium for 3 weeks and then shifted to dehydration desiccation treatment for 72 h. The desiccated calluses were cultured on different media for somatic embryogenesis and plant regeneration. A medium with 2.0 mg/L α-napthaleneacetic acid, 10.0 mg/L abscisic acid , 2.0 mg/L kinetin was best for somatic embryogenesis only, but not for further plant development. After 10 d, differentiated calluses were sub-cultured on medium with various concentrations and types of carbohydrates (carbon source) in $^{1}MS_{2j}$ medium. A large number of plantlets (14.51 ± 2.81 and 8.56 ± 2.90 plants/callus) were regenerated via chemical desiccation, on MS with 3% maltose+3% sorbitol and 6% sucrose, respectively. Under dehydration on only simple MS (3% sucrose), 11.23 ± 3.22 plants/callus were developed. Under conditions of dehydration and chemical desiccation, plant regeneration rates were higher than the calluses cultured on simple MS medium in

Abbreviations: 2,4-D, 2,4-dichlorophenoxy acetic acid; 2-IP, 2-isopentenyl adenosine; Ci, callus induction medium; IAA, indoleacetic acid; KT, kinetin; MS, Murashige and Skoog medium; NAA, α-napthaleneacetic acid; PGR, plant growth regulator; PRM, plant regeneration medium; TDZ, thidiazuron.

*Correspondence to: DMJM International (Cansult Maunsell/AECOM Ltd.), Consultant of Gardens Sector Projects, Alain Municipality and Eastern Emirates, PO Box 1419, Al-Ain, Abu Dhabi, United Arab Emirates.

E-mail addresses: rao.ikram@yahoo.com (Ikram-ul-Haq), abdul79jaleel@yahoo.co.in (C.A. Jaleel).

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the presence of plant growth regulator. After somatic embryogenesis, >25% plants were sterile. The protocol used here may allow maximum regeneration of normal and fertile plantlets of super basmati rice within 3 months.

Introduction

Rice has been a staple food source for more than half of the world's populations since ancient times. It is a member of the world's five most important cereal crops. About 92% of the planet's total rice is produced and consumed in Asia (Khush, 1997). In Pakistan, rice production in 2007 was 5.49 million tons, of which 2.5 million tons were basmati rice (http://www.fao.org/es/esc/en/index.html). Despite its large-scale production, a number of abiotic and biotic factors have been limiting productivity in rice cultivation. Genetic transformation is an invaluable tool to develop natural resistance in plants against all yield-limiting factors, but it depends on an efficient *in vitro* plant regeneration system from a single cell.

Among cereals, the callus initiations in cultured parts (explant) of a species, proliferation, and then subsequent regeneration are prime steps in tissue culturing (Snezana et al., 2005). Each step is to be manipulated by biotechnological means to design an efficient protocol for plant regeneration. The potential for callus formation and regeneration has been reported to be a variety-specific characteristic (Barry-Etienne et al., 2002; El-Bakry and Ahmed, 2002).

Strategies to improve plant regeneration frequency in cereals, including rice, have been steadily evolving during the last decade (Kyozuka et al., 1988; Datta et al., 1992; Raman et al., 1994; Itoh et al., 2006). Different tissues in rice plants have been used as explants (Bhaskaran and Smith, 1988) for callus induction. However, the calluses produced have had limited totipotency for successful regeneration (Maggioni et al., 1989; Vyas et al., 2009), which depends on a number of biophysical characteristics (Korbes and Droste, 2005). During plant regeneration from an embryogenic callus, the somatic embryo is an intermediate stage between the undifferentiated callus (somatic cells) and seedlings. It is a differentiated and meristematic form of a somatic (callus) cell developed through a series of complex morphological and cellular changes (Laux and Jurgens, 1997; Helariutta et al, 2000; George et al., 2008). Specific cellular changes can induce embryo maturation, which is one of the main barriers for the success of somatic embryogenesis (Misra et al., 1993; Tremblay and Tremblay, 1995; Bozhkov and Arnold, 1998; Li et al., 1998; Walker and Parrott, 2001). Biophysical changes in the cells lead to the accumulation of sufficient storage materials (Bozhkov and Arnold, 1998) and desiccation tolerance (Blackman et al., 1992) for conversion to embryos (Fry, 1995; Murthy et al., 1998; Moon and Hildebrand, 2003) and then its maturation (Thomas, 1993; Merkele et al., 1995). Somatic embryogenesis can be influenced even by developing low osmotic potential in the maturation cultures (McKersie and Brown, 1996; Walker and Parrott, 2001). Carbohydrates are commonly used in the cultures as carbon sources for the development of tissues (Iraqi and Tremblay, 2001) into plantlets. These compounds can function in somatic embryogenesis (playing a role as osmotica) and as a nutrition source (Li et al., 1998) in the cultures. In many species, increased sugar concentration generally improves somatic embryo maturation (Tremblay and Tremblay, 1995; Li et al., 1998; Iraqi and Tremblay, 2001). Plant regeneration from embryogenic calluses was initially achieved in *japonica* rice varieties (Nishi et al., 1973). Successful regeneration of fertile plants has been limited in *Indica* rice varieties (Kyozuka et al., 1988; Raman et al., 1994). As a result, progress towards the transfer of useful genes in *Indica* rice has made slow progress.

Normal somatic embryogenesis may occur, but maturation and plant regeneration are dependent on specific physical stresses. Certain stresses may minimize the rate of abnormal plant development. Partial physical desiccation treatments have been reported to be beneficial for embryogenesis and plant regeneration in several plant species (De Gloria et al., 2000; Tyagi et al., 2007; Corredoira et al., 2008; Mingozzi et al., 2009). The dehydration of cell suspension derived from the embryogenic calluses (Tsukahara and Hirosawa, 1992; Jain et al., 1996; Zhu et al., 1996; Chand and Sahrawat, 2001) can increase plant regeneration efficiency. On the basis of this idea, we aimed to observe the effect of chemical desiccation through maltose and sorbitol supplements (to increase osmotic pressure) in place of sucrose in the plant regeneration medium (PRM), with an additional dehydration desiccation treatment on proliferating calluses prior to their regeneration.

In this study, we varied a number of cultures to assess the comparative effect of different

hormones under partial physical desiccation (via both chemical desiccation and dehydration desiccation) stresses on the efficiency of plant regeneration from scutellum-derived embryogenic callus of recalcitrant *Indica* rice variety ''Super basmati''. This study may aid future work to establish genetic transformation systems to improve rice or other cereal crops.

Materials and methods

Plant material and sterilization

Mature healthy seeds of rice (*Oryza sativa* L.) variety super basmati were selected, dehusked and surface sterilized with 50% (1:1, v/v) commercial bleach (5.25% NaOCl) by stirring on a magnetic stirrer for 1 h. They were then washed (3×5 min) with sterile distilled water. Twenty to thirty surface sterilized seeds were cultured on a number of callusing media. The cultures were incubated at $25 \pm 2\,^{\circ}C$ in dark.

Callusing cultures

For callusing, a number of media were maintained, including MS (Murashige and Skoog) basal medium (Murashige and Skoog, 1962) [basal salts, B_5 vitamins (Gamborg et al., 1968) and 3% sucrose] supplemented with 2.0 mg/L 2,4-dichlorophenoxy acetic acid (2,4-D) separately or in a combination with 500 mg/L proline and 2 mg/L α-napthaleneacetic acid (NAA) (Table 1). Each type of medium was solidified with 1% (w/v) purified granulated agar (Difco) and its pH was adjusted between 5.7 and 5.8 prior to sterilization.

Callus proliferation

The callus proliferation rate (%) was measured by culturing ~50 mg callus (after 7-d) from $^{*}MS_{2a}$ (Table 1) to all other media, including itself ($^{*}MS_{2a}$- $^{1}MS_{2j}$) for 3 weeks. It was calculated using the formula:

$$\frac{FW_f - FW_i}{FW_f} \times 100$$

where FW_i was the initial fresh weight (50 mg) and FW_f was the final fresh weight of calluses.

Physical desiccation treatment

Partial physical desiccation was carried out by transferring embryogenic calluses from $^{*}MS_{2a}$ (somatic embryo induction) to sterile empty Petri dishes containing two sterile Whatman^{-1} filter papers for dehydration desiccation. The Petri dishes were sealed with parafilm and kept at $25 \pm 2\,^{\circ}C$ in the dark for 72 h. After desiccation treatment, calluses were transferred to different plant regeneration media (Table 1). For chemical desiccation experiments, sucrose (6%) ('c' in Table 1) and sorbitol (3%)+maltose (3%) ('d' in Table 1) were added in the plant regeneration medium (PRM) in place of 3% sucrose (w/v). The PRM (comprised on MS salts, B_5 vitamins and 3% sucrose) was kept as a control PRM on which embryogenic calluses was cultured without desiccation treatment (culture is shown as 'a' in Table 1). After dehydration desiccation treatment, the calluses were also cultured on PRM ('b' in Table 1).

Plant regeneration

A number of cultures were maintained for plant regeneration (Table 1). One specific culture ($^{1}MS_{2j}$) was also established by culturing calluses from $^{1}MS_{2h}$ (after 10 d). Plant regeneration was observed after 30-d in each of the cultures (see Table 1).

Root induction and plant hardening

The regenerated plantlets were transferred to culture tubes containing $\frac{1}{2}$MS basal medium for shoot elongation and root induction. After 2 weeks, rooted plantlets were transferred to soil in earthen pots covered with polythene bags for plant hardening. The plants were finally shifted to the green house after 7 d.

All cultures were incubated at $25 \pm 2\,^{\circ}C$ under 16/8 h day and light conditions (light intensity 15 μmol m^{-2} s^{-1}) provided by white fluorescent tubes (36 W/54, 6500 K) in the growth room until plant hardening on the soil medium.

Growth regulators sterilization

A number of plant growth regulators (PGRs) were used in all cultures. They were sterilized differently depending upon their stability. For example, the heat labile growth regulators indoleacetic acid (IAA) and abscisic acid (ABA) were filter sterilized by using a sterile Millex-GS, 0.22 μm filter unit, while others, e.g. NAA, 2,4-D, kinetin (KT) and 2-isopentenyl adenine (2-IP), thidiazuron (TDZ) were added in the medium (from stock) before autoclaving.

Table 1. The callusing and plant regeneration from the scutellum-derived embryogenic callus through partial physical desiccation in rice (*Oryza sativa* L.) variety super basmati.

Media	Treatment/ hormones	Callus induction (%)	Callus proliferation rate (%) 3-weeks culture	a. Somatic embryo induction than to sucrose (3%) (plantlets/callus)	Dehydration desiccated embryogenic calluses		
					b. Dehydration	Chemical	
					3% Sucrose (plantlets/callus)	c. 6% Sucrose (plantlets/callus)	d. 3% Sorbitol+3%maltose (plantlets/callus)
Ci							
*MS_{2a}	2,4-D (2 mg/L)	92.0	60.25	–	–	–	–
*MS_{2b}	2,4-D+NAA (2 mg/L)	70.2	32.75	–	–	–	–
*MS_{2c}	2,4-D+proline (500 mg/L)	79.5	56.25	–	–	–	–
PRM							
$^{1}MS_{2d}$	2-IP (2 mg/L)	–	17.40	2.76 ± 0.39	$^{*}6.18 \pm 0.20$	$^{*}4.91 \pm 0.25$	10.21 ± 0.50
$^{1}MS_{2e}$	TDZ (2 mg/L)	–	12.12	1.39 ± 0.35	$^{ns}2.72 \pm 0.25$	$^{ns}2.35 \pm 0.45$	8.11 ± 0.15
$^{1}MS_{2f}$	IAA (2 mg/L)	–	22.56	–	$^{ns}0.34 \pm 0.42$	–	2.52 ± 0.65
$^{1}MS_{2g}$	NAA+KT (2 mg/L each)	–	18.21	2.56 ± 0.26	$^{ns}3.31 \pm 0.35$	$^{ns}3.59 \pm 0.25$	8.42 ± 0.27
$^{1}MS_{2h}$	NAA+KT+ABA (10 mg/L)	–	11.52	–	–	–	–
$^{1}MS_{2i}$	MS basal medium	–	08.51	–	–	–	–
$^{1}MS_{2j}$	MS_{2i} (callus from $^{1}MS_{2h}$ after 10-d	–	69.92	3.65 ± 0.6	$^{**}11.23 \pm 0.15$	$^{*}8.56 \pm 0.29$	14.51 ± 0.28

*, ** letters in the column show significant difference at $p \leqslant 0.05$, while ns is non significant treatment difference. PRM: Plant Regeneration Medium.

Statistical data analysis

The experiment was arranged as a randomized complete block with 7 replicates per treatment during callusing or plant regeneration. Data were analyzed using the SAS program Version 6.11 (SAS Institute, Cary, NC, USA). A probability level of 5% (= 0.05) was chosen for all statistical inferences.

Results and discussion

The yield potential of cereals is currently limited by a number of biotic and abiotic stresses. The present yield of cereals is not fulfilling the increasing demand from human populations. No method is currently available for crop improvement over a very short period of time. Modern biotechnology could enable cereals to resist specific environmental stresses and to increase potential in the form of yield. Genetic engineering is dependent on an efficient plant regeneration system of a crop. The first part of the process is callusing from any tissue (explant) of plant, which is an important step in establishing its tissue culture system.

Here, a number of media were appraised for callus induction and proliferation in rice, including MS supplemented with 2,4-D (2.0 mg/L), NAA (2.0 mg/L) and proline (500 mg/L) separately or in combination (*MS_2). Within 7 d, calluses were induced from the scutellar region of seeds on each medium. Maximum callus induction frequency (92.0%) was observed on the *MS_{2a} medium (Table 1). The callus proliferation rate (%) was also measured by subculturing embryogenic calluses from *MS_{2a} medium to fresh callus induction (Ci) medium and plant regeneration medium (PRM). After 3 weeks, maximum 60.25% callus proliferation was observed on *MS_{2a} (Table 1). The culture with 2,4-D only induced efficient calluses and then proliferation (Figure 1a) in super basmati rice (Katiyar et al., 1999; Zhenyu et al., 1999; Gairi and Rashid, 2004). However, use of casein hydrolysate was found to be beneficial for generation of embryogenic calluses in both *Japonica* (Hiei et al., 1994; Khanna and Raina, 1997; Toki, 1997) and in *Indica* rice varieties (Zhang et al., 1996). Similarly, the use of proline was also effective for the initiation and maintenance of embryogenic calluses (Datta et al., 1992; Kishor et al., 1999).

Partial physical desiccation has been found to promote plant regeneration (Jain, 1997; Diah and Bhalla, 2000; Chand and Sahrawat, 2001; Saharan et al., 2004). Here, plant regeneration in embryogenic calluses was started on $^1MS_{2j}$ culture (Figure 1) within 2 weeks. They were then cultured from $^1MS_{2h}$ (somatic embryo induction) medium (Figure 1b) onto $^1MS_{2j}$ (chemically desiccated plant regeneration) medium. After 2–4 weeks, the plantlets were regenerated on medium on which the cultured embryogenic calluses were treated as the dehydrated desiccation, and after 4 weeks on the medium supplemented with 6% and 3% sucrose (Table 1). With the exceptions of the $^1MS_{2jh}$ and $^1MS_{2i}$ media, the plantlets were regenerated after 7 weeks.

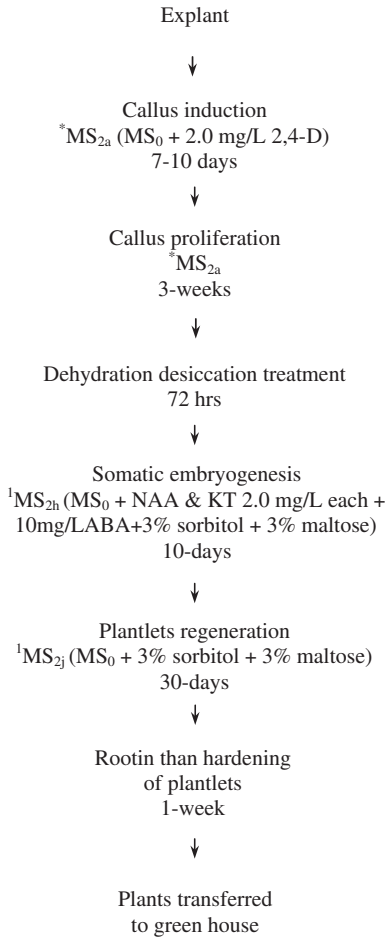

Figure 1. A schematic representation of an efficient plant regeneration (via chemical desiccation) protocol for super basmati rice (*Oryza sativa* L.) variety within 3 months.

Within 4 weeks, the somatic embryogenic calluses on $^1MS_{2j}$ were entirely covered with green shoot buds. Vigorous elongation with efficient shoot multiplication was observed. The shoot multiplication rate was comparatively low in the cultures with sucrose and dehydrated desiccation treatment on $^1MS_{2j}$ relative to cultures with maltose and sorbitol. Similarly, the physical appearance of the regenerated plantlets was less green and healthy

than were the plantlets regenerated from chemical desiccation medium ($^1MS_{2j}$ with maltose and sorbitol). The numbers of regenerated plantlets from a single embryogenic callus were 10.21 ± 4.88 and 14.51 ± 2.81 (maltose and sorbitol), but 6.18 ± 2.11 and 11.23 ± 1.22 (dehydrated desiccation) and 4.91 ± 2.50 and 8.56 ± 2.90 (6% sucrose) on $^1MS_{2d}$ and $^1MS_{2j}$ media, respectively. All of these cultures proved better than cultures maintained on 3% sucrose medium (Table 1). Large numbers of plantlets were obtained by culturing embryogenic calluses on $^1MS_{2h}$ medium for 10 d prior to subculturing on the $^1MS_{2j}$ (Figure 1d) medium. All of these regenerated plantlets from $^1MS_{2f}$ (maltose and sucrose) were also observed to be fully fertile. Few plantlets were regenerated on $^1MS_{2f}$ and $^1MS_{2e}$ (supplemented with TDZ and IAA, respectively), and appeared to be less healthy and fertile.

Overall, partial physical desiccation enhanced plant regeneration efficiency for super basmati rice. Chemical desiccation instead of sucrose in MS cultures proved to be helpful for the improvement of both the maturation of somatic embryos and their regeneration into plantlets. It was also noted that regeneration without somatic embryo induction ($^1MS_{2h}$ with 10 mg/L ABA, NAA, KT, maltose and sorbitol) in calluses is impossible (Figure 1). During somatic embryogenesis in the cultures, growth inhibition, due to ABA (a growth inhibitor), was observed (Figure 2b).

The results of this study show that, in *Indica* rice super basmati, the highest number of plantlets (14.51 ± 2.81) were regenerated (Figure 2c) through chemical desiccation (maltose and sorbitol) treatment in comparison to the plants regenerated after dehydration desiccation or chemical desiccation (6% sucrose) treatment. The desiccation treatments, during or before plant regeneration, were observed to be more effective than callus cultures without desiccation. With applications of desiccation, all the regenerated plantlets were fertile.

The recalcitrance of *Indica* rice varieties to tissue culture has been a major stumbling block for their transgenic development. In addition, the fact that agronomic *Indica* rice improvement currently depends totally on the *Japonica* rice varieties could potentially lead to a genetic bottleneck problem. Using this tissue culture protocol for super basmati rice, we produced a high percentage of regenerable somatic embryogenic calluses, in the presence of a combination of different hormones in the somatic embryogenesis medium and through partial physical desiccation (in the absence of PGR). Both $^1MS_{2h}$ and $^1MS_{2j}$ media (Table 1), in particular, produced excellent results, both for the development of somatic embryos (PGR) and for efficient plant regeneration (partial physical desiccation). However, when plant regeneration was carried through partial physical desiccation in the presence of PGR, $>25\%$ of total regenerated plantlets were sterile.

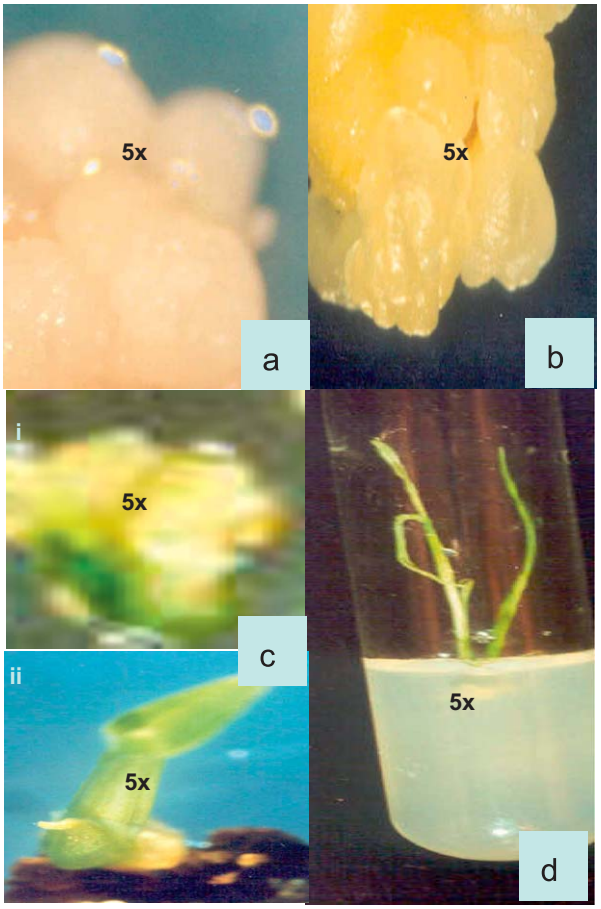

Figure 2. Cultures developed during the establishment of a protocol for plant regeneration in rice (*Oryza sativa* L.) variety super basmati: (a) callus proliferation on the $^*MS_{2a}$ medium; (b) somatic embryogenesis in the embryogenic calli on $^1MS_{2h}$ medium after 10 d of culture; (c) the somatic embryogenesis at different developmental stages with well-developed regenerated plantlet [(i) during the 1st week of culture, callus is going to be greenish after desiccation on $^1MS_{2j}$; (ii) than regenerated plantlets on the same medium after 3rd week]; and (d) root induction in the regenerated plantlets on the MS_0 medium.

We are currently testing the embryogenic potential for plant regeneration efficiency by using the protocol described in this paper for the purpose of establishing its genetic transformation system. Super basmati rice is an agronomically improved cultivar with good yield and is highly palatable, so it will have little genetic drift in transgenic backcross programs as compared to other *Japonica* rice varieties. Thus, regeneration of plants through somatic embryogenesis in super basmati rice

constitutes a significant step towards broadening the genetic base of transgenic rice cultivars.

References

Barry-Etienne D, Bertrand B, Vasquez N, Etienne H. Comparison of somatic embryogenesis-derived coffee (*Coffea arabica* L.) plantlets regenerated *in-vitro* or *ex-vitro*: morphological, mineral and water characteristics. Ann Bot 2002;90:77–85.

Bhaskaran S, Smith RH. Enhanced somatic embryogenesis in *Sorghum biocolor* L. from shoot tip culture. In Vitro Cell Dev Biol 1988;24:65–70.

Blackman SA, Obendorf RL, Leopold AC. Maturation proteins and sugars in desiccation tolerance of developing soybean seeds. Plant Physiol 1992;100: 225–30.

Bozhkov PV, Arnold S. Polyethylene glycol promotes maturation but inhibits further development of *Picea abies* somatic embryos. Physiol Plantarum 1998;104: 211–24.

Chand S, Sahrawat AK. Stimulatory effect of partial desiccation on plant regeneration in *Indica* rice (*Oryza sativa* L.). J Plant Biochem Biotechnol 2001;10:43–7.

Corredoira E, Valladares S, Vieitez AM, Ballester A. Improved germination of somatic embryos and plant recovery of European chestnut. In Vitro Cell Dev Biol-Plant 2008;44:307–15.

Datta SK, Datta K, Soltanifar N, Donn G, Potrykus I. Herbicide-resistant *Indica* rice plants from IRRI breeding line IRRI after PEG-mediated transformation of protoplast. Plant Mol Biol 1992;20:619–29.

De Gloria FJM, Filho FDAM, Mende BMJ. Plant regeneration from protoplast of Brazilian citrus cultivars. Pesq Agropec Bras 2000;35:727–32.

Diah P, Bhalla PL. Plant regeneration from mature embryo derived callus of *Australian* rice (*Oryza sativa* L.) varieties. Aust J Agric Res 2000;51:305–12.

El-Bakry, Ahmed A. Effect of genotype, growth regulators, carbon source, and pH on shoot induction and plant regeneration in tomato. In Vitro Cell Dev Biol-Plant 2002;38:501–7.

Fry SC. Polysaccharide-modifying enzymes in the plant cell wall. Annu Rev Plant Physiol Mol Biol 1995;46: 497–520.

Gairi A, Rashid A. TDZ-induced somatic embryogenesis in non-responsive caryopses of rice using a short treatment with 2,4-D. Plant Cell Tissue Organ Cult 2004;76:29–33.

Gamborg OL, Miller RA, Ojima K. Nutrient requirements of suspension cultures of soybean root cells. Exp Cell Res 1968;50:151–8.

George EF, Hall MA, De-Klerk G. Somatic embryogenesis. In: Plant propagation by tissue culture, 3rd ed. Springer Publishers; 2008. p. 335–54 [Chapter 9].

Helariutta Y, Fukaki H, Wysocka-Diller J, Nakajima K, Jung J, Sena G, et al. The short-root gene controls radial patterning of the *Arabidopsis* root through radial signaling. Cell 2000;101:555–67.

Hiei Y, Ohta S, Komari T, Kumashiro T. Efficient transformation of rice (*Oryza sativa* L.) mediated by *Agrobacterium* sequence analysis of the boundaries of the T-DNA. Plant J 1994;6:271–82.

Iraqi D, Tremblay FM. The role of sucrose during maturation of black spruce (*Picea mariana*) and white spruce (*Picea glauca*) somatic embryos. Physiol Plantarum 2001;111:381–8.

Itoh J, Sato Y, Nagato Y, Matsuoka M. Formation, maintenance and function of the shoot apical meristem in rice. Plant Mol Biol 2006;60:827–42.

Jain RK. Effects of some factors on plant regeneration from *Indica* rice cells and protoplasts. J Exp Biol 1997; 35:323–31.

Jain RK, Jain S, Wang B, Wu R. Stimulatory effect of water stress on plant regeneration in aromatic *Indica* rice varieties. Plant Cell Rep 1996;15:449–54.

Katiyar SK, Chandel G, Singh P, Pratibha R. Genetic variation and effect of 2,4-D in *in-vitro* plant regeneration in *Indica* rice cultivars. Oryza 1999;36: 254–6.

Khanna HK, Raina SK. Enhanced *in vitro* plantlet regeneration from mature embryo-derived primary callus of Basmati rice cultivar throughout modification of nitrate-nitrogen and ammonium-nitrogen concentrations. J Biochem Biotechnol 1997;16:85–9.

Khush GS. Breaking the yield frontier of rice. Geol J 1997;35:329–32.

Kishor PBK, Sangam S, Naidu KP. Sodium, potassium, sugar, alcohol and proline mediated somatic embryogenesis and plant regeneration in recalcitrant rice callus. In: Plant tissue culture biotechnology: emerging trends, Proceedings of the symposium held at Hyderabad, India, 1999. p. 78–85.

Korbes AP, Droste A. Carbon sources and polyethylene glycol on soybean somatic embryo conversion. Pesq Agropec Bras Brasília 2005;40:211–6.

Kyozuka J, Otoo E, Shimamoto K. Plant regeneration from protoplast of *Indica* rice: genotype differences in culture response. Theor Appl Genet 1988;76: 887–90.

Laux T, Jurgens G. Embryogenesis. A new start in life. Plant Cell 1997;9:989–1000.

Li XY, Huang FH, Murphy B, Gbur-junior EE. Polyethylene glycol maltose enhance somatic embryo maturation in loblolly pine (*Pinus taeda* L.). In Vitro Cell Dev Biol-Plant 1998;34:22–6.

Maggioni L, Lusrdi MC, Lupotto E. Effect of culture condition callus induction plant regeneration from mature, immature embryos of rice varieties (*Oryza sativa* L.). J Genet Breed 1989;43:99–106.

McKersie BD, Brown DCW. Somatic embryogenesis and artificial seeds in forage legumes. Seed Sci Res 1996;6:109–26.

Merkele SA, Parrott WA, Flinn BS. Morphogenic aspects of somatic embryogenesis. In: Thorpe TA, editor. *In vitro* embryogenesis in plants. Dordrecht, Boston, London: Kluwer Academic Publishers; 1995. p. 155–203.

Mingozzi M, Montello P, Merkle S. Adventitious shoot regeneration from leaf explants of eastern cottonwood

(*Populus deltoides*) cultured under photoautotrophic conditions. Tree Physiol 2009;29:333–43.

Misra S, Attree SM, Leal I, Fowke LC. Effect of abscisic acid, osmoticum, and desiccation on synthesis of storage proteins during the development of white spruce somatic embryos. Ann Bot 1993;71:11–22.

Moon H, Hildebrand DF. Effects of proliferation, maturation, and desiccation methods on conversion of soybean somatic embryos. In Vitro Cell Dev Biol-Plant 2003;39:623–8.

Murashige T, Skoog F. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol Plant 1962;15:473–97.

Murthy BNS, Murch SJ, Saxena PK. Thidiazuron: a potent regulator of *in vitro* plant morphogenesis. In Vitro Cell Dev Biol-Plant 1998;34:267–75.

Nishi T, Yamada Y, Takahishi E. The role of auxins in differentiation of rice tissue culture *in vitro*. Bot Mag (Tokyo) 1973;86:183–8.

Raman R, Chahal GS, Dhaliwal HS. Screening of genotype for callus induction and plant regeneration in rice. Crop Improv 1994;21:1–2.

Saharan V, Yadav RE, Yadav NR, Chapagain P. High frequency plant regeneration from desiccated calli of *Indica* rice (*Oryza sativa* L.). Afr J Biotechnol 2004; 3:356–9.

Snezana D, Ivic-Haymes, Smigocki AC. Identification of highly regenerative plants within sugar beet (*Beta vulgaris* L.) breeding lines for molecular breeding. In Vitro Cell Dev Biol-Plant 2005;41:483–8.

Thomas TL. Gene expression during plant embryogenesis and germination: an overview. Plant Cell 1993;5: 1401–10.

Toki S. Rapid and efficient *Agrobacterium*-mediated transformation in rice. Plant Mol Biol Rep 1997;15:16–21.

Tremblay L, Tremblay FM. Maturation of black spruce somatic embryos: sucrose hydrolysis and resulting osmotic pressure of the medium. Plant Cell Tissue Organ Cult 1995;42:39–46.

Tsukahara M, Hirosawa T. Simple dehydration treatment promotes plantlets regeneration of rice (*Oryza sativa* L.) callus. Plant Cell Rep 1992;13:647–65.

Tyagi RK, Agrawal1 A, Mahalakshmi C, Hussain Z, Tyagi H. Low-cost media for *in vitro* conservation of turmeric (*Curcuma longa* L.) and genetic stability assessment using RAPD markers. In Vitro Cell Dev Biol-Plant 2007;43:51–8.

Vyas S, Guha S, Bhattacharya M, Rao IU. Rapid regeneration of plants of *Dendrobium lituiflorum* Lindl. (Orchidaceae) by using banana extract. Sci Hortic 2009;121:32–7.

Walker DR, Parrott WA. Effect of polyethylene glycol and sugar alcohols on soybean somatic embryo germination and conversion. Plant Cell Tiss Organ Cult 2001; 64:55–62.

Zhang S, Chen L, Rongda QU, Marmey P, Beachy R, Fauquet C. Regeneration of fertile transgenic *Indica* rice plants following microprojectile transformation of embryogenic suspension culture cells. Plant Cell Rep 1996;15:465–9.

Zhenyu G, Gaozy HD, Huang DN. Some factors influencing callus formation and plant regeneration in *Indica* rice varieties. Plant Physiol Comm 1999;35:113–5.

Zhu Y, Ouyang W, Li Y, Chen Z. The effects of 2ip and 2, 4-D on rice calli differentiation. Plant Growth Regul 1996;19:19–24.