ELSEVIER SCIENTIFIC PUBLISHERS IRELAND

Plant Science 93 (1993) 117–120

PLANT SCIENCE

Genotype-specific optimization of plant regeneration from somatic embryos of soybean

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(Received 19 March 1993; revision received 24 May 1993; accepted 1 June 1993)

Abstract

Proliferative embryogenic suspension cultures were established from immature cotyledon explants of Hartz 'H7190', a cultivar adapted to growing conditions in the southern USA. Germination frequency of somatic embryos of H7190 was low relative to that of PI 417138, a genotype known to have a high germination capacity. Of those H7190 somatic embryos that germinated, the quality of germinants was poor and no plants were recovered. To optimise germination and plant recovery of H7190 somatic embryos, the effect of relative humidity during desiccation treatment was tested. Desiccation in an atmosphere of 85% relative humidity resulted in higher germination frequencies and higher quality germinants than those in other treatments. The optimal desiccation treatment for somatic embryos of H7190 did not improve germination of somatic embryos of PI 417138, 'Peking' or 'Century,' indicating that no current protocol is optimal for plant recovery from all soybean genotypes.

Key words: Somatic embryogenesis; Conversion; Desiccation; *Glycine max*

1. Introduction

Several protocols for regeneration of soybean plants via somatic embryogenesis have been documented [1–4], and proliferative embryogenic cultures amenable to genetic transformation by microprojectile bombardment have been developed. These cultures are maintained and propagated at a globular stage of development by continuous subculture on medium containing 2,4-D [4]. The proliferative quality of these cultures allows for the preferential growth of transformed tissue on selective medium [5]. Proliferative embryogenic cultures are also potentially useful for basic studies of embryo development, production of novel variants, and in vitro selection.

Efficient plant regeneration of soybean via somatic embryogenesis has required sequential culture treatments that result in the differentiation of cotyledon stage somatic embryos and their maturation, germination, and conversion to plants. Factors which have influenced plant recovery include the length of exposure of explants to auxin [3], plant genotype [2,6,7], somatic embryo morphology [1,8], extent of hypocotyl elongation [9], extent of cotyledon development [1] and a partial desiccation treatment prior to germination

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0168-9452/93/$06.00
SSDI 0168-9452(93)03670-Q

[1,3,10]. The importance of these factors has been determined using somatic embryos derived from various protocols. Relatively little is known of factors which influence regeneration from the proliferative embryogenic cultures described by Finer and Nagasawa [4]. Also, studies of soybean regeneration and somatic embryogenesis have primarily used genotypes from early maturity groups, which represent germplasm different from that of the later maturity groups [11].

The objectives of this study were (i) to optimize plant recovery from proliferative embryogenic suspension cultures of Hartz H7190, a cultivar of Maturity Group VII from which plant regeneration was difficult using published protocols, and (ii) to determine the effect of the optimal treatment for H7190 on plant recovery from other genotypes.

2. Materials and methods

2.1. Experimental material

Proliferative embryogenic cultures of H7190 (Maturity Group VII), PI 417138 (Group II), Peking (Group IV), and Century (Group II) were initiated and maintained as liquid shake cultures on Finer and Nagasawa medium [4]. Differentiation and maturation of cotyledon stage somatic embryos was accomplished as previously described [7]. Somatic embryos of ≥ 3 mm in length with one or more well-formed cotyledons and a distinct hypocotyl-root axis were used in all experiments.

2.2. Initial assessment of germination from somatic embryos of H7190

Mature somatic embryos of H7190 and PI 417138 were partially desiccated for 5 days in 100 × 15 mm Petri dishes which contained a 1 cm^3 portion of MSM6 medium [7] placed adjacent to the inner wall of the dish. Petri dishes were sealed with two layers of Nescofilm (Karian Research Products Corporation, Santa Rosa, CA). Somatic embryos were transferred to MSO medium [7] in which viability and germination were scored after 25 days. Germination was defined as the emergence from the shoot apical region of a pubescent structure at least 1 cm in length.

2.3. Optimization

Saturated salt solutions were prepared to provide atmospheres of defined relative humidity (RH). The solutions and RH at 25°C were KNO_3 (93%), KCl (85%), NaCl (75%), NH_4NO_3 (63%) and $MgCl_2$ (33%). Fifty ml of solution prepared as a sludge were dispensed into sterile Magenta GA-7 boxes (Magenta Corp., Chicago, IL). A high humidity chamber (~100%) was prepared by lining the inside of a GA-7 box with a water-soaked paper towel. For each treatment, 35 somatic embryos were transferred to unwrapped 60 × 15 mm Petri dishes. The dishes were placed on the surface of the salt sludge, and the GA-7 box wrapped with two layers of Nescofilm. As a control treatment, 35 somatic embryos were incubated for 5 days in a 100 × 15 mm Petri dish which contained a 1 cm^3 portion of MSM6 medium placed adjacent to the inner wall of the dish as described. After 5 days of desiccation treatment, somatic embryos were transferred to MSO medium and evaluated for viability and germination. Seven replicates of 35 somatic embryos for each treatment were tested. Data were analyzed using SAS PROC GLM and mean separations were made with Fisher's Protected LSD. Sixty vigorous germinants derived from somatic embryos which had been desiccated in an atmosphere of 85% RH for 5 days were evaluated for conversion to plants as described in Bailey et al. [7].

2.4. Control treatment vs. 85% relative humidity: PI 417138, Peking, and Century

Since 85% RH promoted germination and plant recovery of H7190 somatic embryos, the control and 85% desiccation treatments were compared for effects on germination of somatic embryos of Century, Peking, and PI 417138. Germination was defined as described previously, and final germination frequency was scored after 25 days on MSO medium.

3. Results and discussion

A broad definition of germination, i.e., the emergence of pubescent shoot structures of at least 1 cm in length after 25 days on medium, was used in these experiments since the responses of individual somatic embryos after transfer to germination medium were unique and defied rigid classification. The quality of apical structures

ranged from pubescent leaf-like structures to elongated, vigorous plants with several trifoliate leaves.

In a preliminary experiment, germination frequency of H7190 (11% ± 2%) was approx. 3-fold lower than that of PI 417138 (37% ± 3%). Germinants of PI 417138 resembled seed-derived plants whereas those of H7190 were stunted apical structures without expanded foliage leaves. Furthermore, upon transfer to soil, no plants were recovered from germinants of H7190, while most germinants of PI 417138 survived and continued growth.

The germination frequency of H7190 somatic embryos was different among RH treatments ($P < 0.0001$; Fig. 1), and the highest germination frequency was observed following treatment at 85% RH. In addition, many of the germinants from this treatment had elongated hypocotyls and expanded foliage leaves. Fifty-eight of sixty vigorous germinants from the 85% RH treatment survived and continued growth in soil. Thus, desiccation at 85% RH for 5 days and selection of high-quality germinants enabled plant recovery frequencies of almost 100%.

Somatic embryos of PI 417138, Peking, and Century have, a high, medium, and low relative capacity for germination respectively [7], and were, therefore, used to evaluate the universality of the effect of 85% RH treatment on germination. Although a trend for higher germination frequency after treatment at 85% RH was apparent, desiccation treatment had no significant effect on germination frequency from any of these three genotypes ($P < 0.05$; Fig. 2), nor was there a genotype × desiccation treatment interaction. Germination frequency was different among these three genotypes ($P < 0.01$; Fig. 2), and genotype rankings were identical to those reported previously [7].

The results of this study demonstrate that no current protocol is optimal for the recovery of all soybean genotypes. These results with RH treatments are in general agreement with those of Buchheim et al. [1], who found that desiccation of somatic embryos of 'Gem' at 75% RH for 4 or 7 days more effectively promoted conversion than desiccation treatments at 40% (ambient) or 93% RH. The desiccation treatment used by Parrott et al. [3] and the 40% RH treatment described by Buchheim et al. [1] consisted of incubating somatic embryos in sealed Petri dishes with no medium. Using this protocol, Parrott et al. [3] documented germination frequencies following a 1-week desiccation treatment of 26–100%, depending on genotype, whereas Buchheim et al. [1] found that no somatic embryos of 'Gem' survived after 4 days of this desiccation treatment. The reason for this disparity may be due to genotype differences, the length of desiccation period, or to differences in the regeneration protocols used. A simpler possibility may be related to the number, size

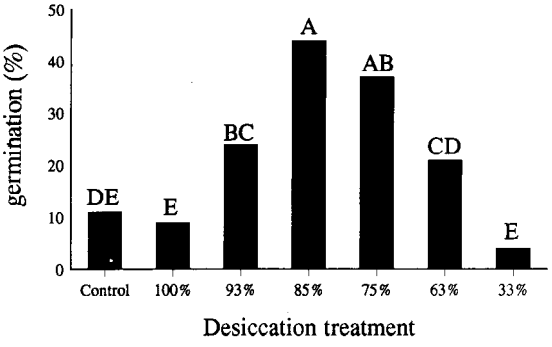

Fig. 1. Mean % germination of H7190 somatic embryos following desiccation treatment at various relative humidities. Data were transformed by arcsin $y^{-0.5}$ for analysis of variance and for mean separation by Fisher's Protected LSD. Bars of the same pattern with different letters are significantly different at $P < 0.05$.

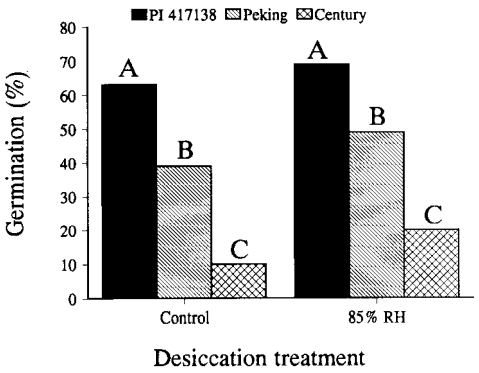

Fig. 2. Mean % germination of three genotypes after two desiccation treatments. Desiccation treatments were not different for germination or conversion frequency. Bars with different letters are different at $P < 0.01$.

and/or extent of hydration of somatic embryos transferred to dry Petri dishes for desiccation. Condensation builds up on the inside of dry, sealed Petri dishes as a result of evaporation from hydrated somatic embryos. Thus, the relative humidity of this treatment is unknown and does not necessarily translate to ambient humidity as indicated by Buchheim et al. [1]. In order to maintain a high relative humidity within the Petri dish regardless of the number and size of somatic embryos, the method of Parrott et al. [3] was modified by including a clump of medium within otherwise dry Petri dishes. This treatment was used as a control in this study since a similar protocol was previously used effectively for germination and conversion of somatic embryos from proliferative embryogenic cultures [7].

In conclusion, the use of an 85% RH treatment promoted high-frequency germination and subsequent plant recovery from somatic embryos of H7190 relative to that observed with previously described protocols (i.e. the control treatment), while not affecting germination frequency from somatic embryos of PI 417138, Peking, and Century. These genotype-specific results indicate that it may be necessary to optimize protocols to achieve acceptable frequencies of plant regeneration from somatic embryos of a given soybean genotype.

4. Acknowledgements

This research was supported by a grant from Pioneer Hi-Bred International. The assistance of C. Chlebnikow is greatly appreciated.

5. References

1 J.A. Buchheim, S.M. Colburn and J.P. Ranch, Maturation of soybean somatic embryos and the transition to plantlet growth. Plant Physiol., 89 (1989) 768–775.
2 T. Komatsuda and K. Ohyama, Genotypes of high competence for somatic embryogenesis and plant regeneration in soybean *Glycine max*. Theor. Appl. Gen., 75 (1988) 695–700.
3 W.A. Parrott, G. Dryden, S. Vogt, D.F. Hildebrand, G.B. Collins and E.G. Williams, Optimization of somatic embryogenesis and embryo germination in soybean. In Vitro Cell. Dev. Biol., 24 (1988) 817–820.
4 J.J. Finer and A. Nagasawa, Development of an embryogenic suspension culture of soybean (*Glycine max* Merrill.). Plant Cell Tiss. Org. Cult., 15 (1988) 125–136.
5 J.J. Finer and M.D. McMullen, Transformation of soybean via particle bombardment of embryogenic suspension culture tissue. In Vitro Cell. Dev. Biol., 27 (1993) 175–182.
6 W.A. Parrott, E.G. Williams, D.F. Hildebrand and G.B. Collins, Effect of genotype on somatic embryogenesis from immature cotyledons of soybean. Plant Cell Tiss. Org. Cult., 16 (1989) 15–21.
7 M.A. Bailey, H.R. Boerma and W.A. Parrott, Genotype effects on proliferative embryogenesis and plant regeneration of soybean. In Vitro Cell. Dev. Biol. (1993) in press.
8 P.A. Lazzeri, D.F. Hildebrand and G.B. Collins, A procedure for plant regeneration from immature cotyledon tissue of soybean. Plant Mol. Biol. Rep., 3 (1985) 160–167.
9 T. Komatsuda, W. Lee and S. Oka, Maturation and germination of somatic embryos as affected by sucrose and plant growth regulators in soybeans *Glycine gracilis* Skvortz and *Glycine max* (L.) Merr. Plant Cell Tiss. Org. Cult., 28 (1992) 103–113.
10 N. Hammatt and M.R. Davey, Somatic embryogenesis and plant regeneration from cultured zygotic embryos of soybean (*Glycine max* L.). J. Plant Physiol., 128 (1987) 219–226.
11 X. Delannay, D.M. Rodgers and R.G. Palmer, Relative genetic contributions among ancestral lines to North American soybean cultivars. Crop Sci., 23 (1983) 944–949.