International Scholars Journals

Frontiers of Agriculture and Food Technology Vol. 2 (1), pp. 001-004, January, 2012. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Short Communication

Callus induction and growth in transgenic potato genotypes

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Accepted 22 October, 2011

The aim of this study was to establish an effective protocol for callus induction from the potato genotypes (*Solanum tuberosum* L.) used and to investigate whether the transferred oxalate oxidase enzyme or transformation procedure has any effect on callus induction of transgenic lines of cultivar Desiree and Maris Bard. The results showed that the effects of genotype and medium on measured characters were highly significant. In order to compare overall performance of transgenic lines of each cultivar with their parental cultivars (non-transgenic), orthogonal comparisons were used and indicated the significant differences between transgenic and non-transgenic genotypes. The significant differences between the cultivars and their transgenic lines expressing oxalate oxidase enzyme indicated that the transformation procedure had a significant effect on their callus induction and growth.

Key words: Callus, Oxalate oxidase, solanum tuberosum, transformation.

INTRODUCTION

Much work has been carried out on callus induction and growth in potatoes (*Solanum tuberosum* L.). This has resulted in a range of protocols and procedures being established by researchers since tissue culture gained an importance in plant propagation, conservation and breeding (Ahloowalia 1982; Wareh et al., 1989). Previous researches showed that media used for callus induction and growth depends on the genotypes Gonzalez *et al*. (2001, Alexeenko and Irkaeva (1998) pointed out that introduction of genes effecting the structure and type of plant development into strawberry lines also influenced callus formation and shoot inducing *in vitro*. This depicts involvement of inheritance in callus growth.

In the last two decades, many transgenic plants have been produced by using a range of transformation methods.

Callus is used for most of these transformation methods such as particle gun (McCabe et al., 1998) and *Agrobacterium tumefaciens*-mediated transformation (Stiekema et al., 1988) as well as initiation of cell culture. A callus from an explant tissue occurs as a result of dramatic changes in the appearance and metabolism of the cells (Aitchison et al., 1978). Induction of callus, physical disorganization of cultured cells, is thought as result of the breakdown of intercellular physical and chemical communication (Lindsey and Jones, 1992). In this transportation system, vascular bundles in a plant play important roles in terms of transferring water and plant nutrients. Recently, some enzymes such as oxalate oxidase, which is more expressed in salt tolerant species, are thought to have an effect on the vascular system (Hurkman and Tanaka, 1996).

Therefore, the aim of this study was to establish an effective protocol for callus induction from the potato genotypes. Secondly, it was to investigate whether the genetic transformation procedure and oxalate oxidase

Table 1. Formulation of three media used for callus induction and growth.

Medium 1	Medium 2	Medium 3
MS salts	MS salts	MS salts
MS vitamins	MS vitamins	MS vitaminszz
Sucrose (30 gl^{-1})	Sucrose (30 gl^{-1})	Sucrose (30 gl^{-1})
Zeatin (5 mgl^{-1})	Kinetin (0.5 mgl^{-1})	Kinetin (0.5 mgl^{-1})
NAA (0.1 mgl^{-1})	NAA (5 mgl^{-1})	2,4-D (3 mgl^{-1})
Difco-Bacto Agar (8 gl^{-1})	Difco-Bacto Agar (8 gl^{-1}	Difco-Bacto Agar (8 gl^{-1}
pH 5.8	pH 5.8	pH 5.8

transgene have any effect on callus induction in potato genotypes.

MATERIALS AND METHODS

Two potato cultivars (Maris Bard and Desire) were obtained from the collections of Department of Agricultural Botany at The University of Reading, United Kingdom. Three transformed genotypes (MB_{T1}, MB_{T2} and MB_{T3}) were derived from cultivar Maris Bard (MB), and one (DS_{T1}) derived from cultivar Desiree by using *A. tumefaciens*-mediated transformation method (Turhan, 1997). Transformed genotypes expressing oxalate oxidase enzyme were initially conformed by using kanamycin resistance as the selective marker and the oxalate oxsidase enzyme activity as the reporter gene support the expression of transgene. All genotypes were grown on Murashige and Skoog (1962) basal culture medium without any growth regulators.

Several plant parts such as leaf, root, stem and tuber have been used successfully for callus induction in potato (Ahloowalia, 1982; Austin, 1989; Osifa, 1989). In our case, stem segments were chosen as primary studies showed that stem section was the most responsive explant for the cultivars. The stem segments, approximately 0.5 cm long and uniform diameter (approximately 1 mm), were excised from four week old *in vitro* plantlets. Excised stems without axillary buds, for each genotype, were collected in a Petri dish containing sterile water to randomise the explants before transferring to the media.

The major growth regulators that have been used successfully in callus induction and growth are kinetin, 2, 4-dichlorophenoxy acetic acid (2,4-D), α-naphthaleneacetic acid (NAA), indole-3-acetic acid (IAA), zeatin and gibberellic acid (Osifa,1989; Szabo *et al*., 1994). Therefore, three different modified callus induction media based on the basal MS medium and vitamins were used (Table 1). After preparation of the media, Petri dishes were divided into two equal parts by drawing a middle line. Each half of the Petri dishes was inoculated with four stem segments.

After incubation of stem segments in darkness for four weeks, several characteristics were recorded .These include

Callus size (mm): Mean diameters of callus formation from two ends of a stem segment.

Callus type: It was scored as no callus (0), compact (1), intermediate (2) and friable (3).

Callus colour: 0-9 scale was used for scoring colour of a callus. Brown (0), dark green (1-2), green (3-4), light green (5-6), very light green (7-8) and yellowish green (9).

The number of shoots and roots: Shoots and roots arising from a single explant were counted (if any). This character was not subjected to statistical analysis as few explants had shoots and roots and it is also not desired in callus culture.

The experimental design was a randomised block factorial design with six replicates. Analyses were carried out for these characters on transformed data. The statistical analysis was carried out by using the SAS computer package (SAS Inst. 1998).

RESULTS AND DISCUSSION

In order to evaluate effect of oxalate oxidase enzyme on callus induction and growth, the results were recorded for stem segments from the transgenic and non-transgenic potato genotypes growing on a basal MS medium supplemented with three different combinations of growth regulators. The data were analysed and the analysis showed that the effect of genotype and medium on characters measured was found to be highly significant (Table 2).

In order to compare overall performance of transgenic lines of each cultivar with their parental cultivars (non-transgenic), orthogonal comparisons were used and indicated the significant differences between transgenic and non- transgenic in terms of some characters measured (Table 2).

In media M2 and M3, none of calli had shoots whereas few shoots were observed in M1. However, the root and shoot data were not included since our first aim was not regeneration. In addition, there were no roots in M1 and when the cultures were kept more than four weeks. Few and short roots occurred in M2 and M3.

When the media were compared in terms of overall scores of all genotypes, the calli on M2 were bigger and had growth better than those on M1 and M2. At the same time, in terms of callus quality it varied from ‘intermediate’ to ‘friable’ in type and produced roots (Although the roots were not very long, and less than 1 cm). On the other hand, the calli on M1 tended to produce shoots. After sub-culturing of calli on M2 for bulking purposes, a dramatic reduction was observed in the number of roots. As a result, the best medium for callus induction was M2 (Table 3)

The significant differences between transgenic and non-transgenic (parent) genotypes indicate that transformation procedure had an effect on callus induction. A number of research projects have indicated the relationship between the oxalate oxidase activity of

Table 2. Analysis of variance for characters measured (mean square).

Sources	df	Size	Type	cr
Genotype	5	18.16***	0.33 **	13.04 ***
MB vs MB_{T1} & MB_{T2} & MB_{T3}	1	12.44 ***	0.01	.08 *
DS vs DS_{T1}	1	5.12 **	0.04	0.01
c	2	86.71 ***	16.22 ***	337.13 ***
Genotype x Medium	10	0.54	0.75 ***	7.12 ***
Replication	5	0.59	0.04	1.94
Error	85	0.56	0.10	1.20

*, ** and ***, Significant at 0.05, 0.01 and 0.001 level, respectively.

Table 3. Effect of different media on callus induction and growth in potato genotypes.

Medium	MB	MB_{T1}	MB_{T2}	MB_{T3}	DS	DS_{T1}	Mean
Callus size (mm)($LSD_{0.05}$): Medium = 0.35, Genotype = 0.56							
M1	4.88	3.79	3.96	2.77	5.61	4.92	4.31 c*
M2	7.61	6.94	7.75	5.69	8.42	7.61	7.34 a
M3	5.15	4.91	4.98	3.52	6.87	6.07	5.25 b
Callus type ($LSD_{0.05}$): Medium = 0.14, Genotype = 0.20							
M1	1.00	0.88	1.00	0.83	1.13	1.08	0.99 c
M2	2.08	2.71	2.46	2.58	2.08	2.04	2.33 a
M3	1.63	1.79	1.13	0.96	2.08	1.96	1.59 b
Callus colour ($LSD_{0.05}$): Medium = 0.51, Genotype = 0.72							
M1	1.17	1.58	1.71	1.50	2.08	2.29	1.72 c
M2	6.75	8.83	7.92	7.83	7.83	7.83	7.83 a
M3	3.17	4.38	2.42	3.13	7.00	6.83	4.49 b

*: Means with the same letter in a line are not significantly different at p=0.05.

germin and osmotic and salinity stress in barley and wheat plants (Hurkman et al., 1991; Hurkman, 1992; Lane et al., 1993; Lane, 1994; Hurkman and Tanaka, 1996). At the same time, Zhang *et al.* (1995) pointed out that oxalate oxidase enzyme in barley degrades oxalate produced in fungal infected sites of plant tissue. The degradation process of oxalate gives H_2O_2 and CO_2 (Thompson et al., 1995).

Similarly, Ca^{2+} and H_2O_2 can be released by degradation of calcium oxalate by oxalate oxidase which is in extracellular matrix (Lane, 1994). It can be speculated that the enzyme itself and its products or both may play important roles in the cell wall matrix in terms of water transport (Campbell and Sederoff, 1996), ion selectivity (Showalter, 1993) and cell wall lignification (Halliwell, 1978; Goldberg et al., 1987) by cross linking reaction in higher plants. Moreover, Lindsey and Jones (1992) stated that induction of callus or physical disorganization of cultured cells is thought as a result of the breakdown of intercellular physical and chemical communication. Therefore, the presence of oxalate dase

In potato genotypes might have increased communication of the cells within a callus. Consequently, callus induction and growth in transgenic lines decreased when compared to calli of non-transgenic genotypes (Table 3).

In conclusion, the differences between the cultivars and their transgenic lines in terms of callus growth indicated that transformation procedure or the oxalate oxidase enzyme had a role in this complex mechanism. However, further investigation is required to determine whether this effect comes from transformation procedure alone or oxalate oxidase enzyme.

ACKNOWLEDGMENTS

I would like to thank Prof. P.D.S. Caligari and Dr. A.C. Wetten for their supervision and advice.

REFERENCES

Ahloowalia BS (1982). Plant regeneration from callus culture in potato. Euphytica 31: 755-759.

Aitchison PA, MacLeod AJ, Yeoman MM (1978). Growth patterns in tissue (callus) cultures. In HE Street, ed, Plant Tissue and Cell Culture, Blackwell Sci. Pub. Oxford, pp. 267-306.

Alexeenko TV, Irkaeva NM (1998). Investigation of callus formation and shoot-inducing processes in strains and F1 interstrain hybrids of strawberry *Fragaria vesca* L. Genetika 34(8): 1100-1105.

Austin S, Cassels AC (1989). Variation between plants regenerated from individual calli produced from separated potato stem callus cells. Plant Sci. Lett. 31: 107-114.

Campbell MM, Sederoff RR (1996). Variation in lignin content and composition: Mechanisms of control and implications for the genetic improvement of plants. Plant Physiol. 110: 3 -13.

Fennell S, Bohorova N, Van Gindel M, Crossa J, Hoisington D (1996). Plant regeneration from immature embryos of 48 elite CIMMTY Bread wheats. Theoretical and Appl. Genetics. 92: 163-169.

Goldberg R, Liberman M, Mathieu C, Pierron M, Catesson AM (1987). Development of epidermal cell wall peroxidases along the Mung bean hypocotyls: Posibble involment in the cell wall stiffening process. J. Experimental Botany 38(193): 1378-1390.

Gonzalez JM, Friero E, Jouve N (2001). Influence of genotype and culture medium on callus formation and plant regeneration from immature embryos of Triticum turgidum Desf. Cultivars. Plant Breeding 120(6): 513-517.

Halliwell B (1978). Lignin synthesis: The generation of hydrogen peroxide and superoxide by horseradish and its stimulation by manganese (II) and phenols. Planta. 140: 81-88.

Hurkman WJ (1992). Effect of salt stress on germin expression: A review. Plant and Soil 146: 145 -151.

Hurkman WJ, Tanaka CK (1996). Effect of salt stress on germin gene expression in barley roots. Plant Physiol. 110: 971- 977.

Hurkman WJ, Tao HP, Tanaka CK (1991). Germin-like polypeptides increase in barley roots during salt stress. Plant Physiol. 97: 366-374.

Lane BG (1994). Oxalate, germin, and the extracellular matrix of higher plants. The FASEB Journal. 9: 294-301.ay JA, Schemitt MR, Cumingnt, is an oxalate oxidase. J. Biol. Chem. 268: 12239-12242.

Lindsey K, Jones MGK (1992). Plant Biotechnol. in Agric. John Willey and Sons Ltd., England p. 241

McCabe DE, Swain WF, Martinell BJ, Christou P (1988). Stable transformation of soybean (*Glycine max*) by particle acceleration. Biotechnol. 6: 923-926.

Murashige T, Skoog TF (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiolgia Plantarum. 15: 473-479.*

Osifa EO, Webb JK, Henshaw GG (1989). Variation amongst callus-derived potato plants, Solanum brevidens. Journal of Plant Physiol. 134: 1-4.

SAS Institute (1998) Statistical Analysis System User's Guide: Statistics. SAS Inst., Cary, NC.

Showalter AM (1993). Structure and function of plant cell wall proteins. The Plant Cell 5: 9-23.

Stiekema WJ, Heidekamp F, Louwerse JD, Verhoeven HA, Dijkhuis P (1988). Introduction of foreign genes into potato cultivars Bintje and Desiree using an Agrobacterium tumefaciens binary vector. Plant Cell Reports 7: 47-50.

Szabo M, Koves E, Somogyi I (1994). Development of auxin autotrophy in Nicotiana tabaccum callus cultures. Physiologia Plantarum. 90: 348-352.

Thompson JA, Dunwell JM, Jhonstone CE, Lay V, Schmitt M, Watson H, Nisbet G (1995). Degradation of oxalic acid by transgenic plants expressing oxalate oxidase. Euphytica 85: 169-172.

Turhan H (1997). Salinity studies in potato *Solanum tuberosum* L. Ph.D. Thesis. The University of Reading, UK. p. 252.

Wareh HA, Trolinder NL, Gooding JR (1989) . Callus initiation, shoot regeneration, and micropropagation of three potato cultivars. Hortsci. 24(4): 680-682.

Zhang Z, Collinge DB, Christensen HT (1995). Germin- like oxalate oxidase, a H_2O_2 providing enzyme, accumulates in barley attacked by the powdery mildew fungus. Plant J. 8(1): 139-145.