

The Effect of Pretreating Seedlings with TDZ on Direct Shoot Regeneration from Petiole Explants of Sugar Beet (*Beta vulgaris* L.)

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Abstract. A 100% seed sterility and 75.6% seed germination rate were achieved when seeds of three sugar beet (*Beta vulgaris* L.) breeding lines were surface sterilized with a combination of 0.5% Domestos and 5% PPMTM. The procedures we describe here are more successful than the previously published procedures as a high rate of sterility was achieved without reducing the germinability of sugar beet seeds. As an *in planta* treatment with TDZ (thidiazuron), the sterilized seeds were germinated on MS basal medium containing 1, 3 or 5 mg/L TDZ for five weeks and then petiole explants were excised from the seedlings and cultured on two different regeneration media; RGL medium containing 0.5 mg/L BAP and 0.1 mg/L NAA, and RGH medium containing 1.0 mg/L BAP and 0.5 mg/L NAA. Petiole explants taken from seedlings pretreated with 1 mg/L TDZ produced significantly more shoots (1.67 shoots per explant) than those taken from seedlings pretreated with 3 or 5 mg/L TDZ (1.16 and 0.92 shoots per explant, respectively). The RGL medium containing lower BAP and NAA was significantly more effective in direct shoot production than the RGH medium containing higher BAP and NAA, 1.54 shoots per explant compared to 0.96, respectively. Such an increase in regeneration capacity of the explants due to the TDZ pretreatment is clearly significant for sugar beet, which is regarded as a recalcitrant species. 42.6% of the regenerated shoots developed roots on medium containing 3 mg/L NAA whereas only 19.3% of the shoots rooted on medium containing 1 mg/L NAA. Genotypic variation was significantly evident for both shoot regeneration and rooting.

Keywords. direct shoot regeneration, germination, medium, seed sterilization, sugar beet, TDZ pretreatment.

INTRODUCTION

Sugar beet (*Beta vulgaris* L.) is the most important sugar producing crop in Europe and it supplies about 40% of the world total sugar production (Atanassov, 1986). Breeding of sugar beet is mainly carried out conventionally but in the past couple of decades, the use of molecular techniques, more specifically genetic transformation technologies, has drastically increased (D'Halluin et al., 1992; Trifonova and Atanassov, 1995; Krens et al., 1996; Cai et al., 1997; Mannerlof et al., 1997; Ivic et al., 2001). The development of an efficient protocol for plant regeneration from cultured explants is a prerequisite for the genetic manipulation and improvement studies. Sugar beet is known to be a recalcitrant species with respect to *in vitro* culture and genetic transformation (Tetu et al., 1987; Elliott et al., 1996; Krens et al., 1996). Adventitious shoot regeneration from several cultured explants has been often employed for the propagation of elite genotypes (Detrez et al., 1988; Ritchie et al., 1989; Zhong et al., 1993; Grieve et al., 1997) but there was a high degree of variability in the regeneration frequencies from various explants of different genotypes (Jacq et al., 1992; Zhong et al., 1993; Saunders and Tsai, 1999).

The effects of pretreating explants or seedlings on subsequent shoot production frequencies were previously examined in sugar beet (Zhong et al., 1993; Krens et al., 1996; Zhang et al., 2001) but the results are rather inconsistent, most probably due to the genotypic variation of the material used. In this study, the effect of *in planta* TDZ treatment on adventitious shoot regeneration from petiole explants excised from the treated seedlings was investigated with the aim of improving the procedures for regeneration of some valuable sugar beet breeding material.

MATERIALS AND METHODS

The seeds of three sugar beet (*Beta vulgaris* L.) breeding lines (*M114*: diploid monogerm O-type, good root yield, medium sugar yield; *M1017*: diploid monogerm, normal root yield, normal sugar yield; *ELK345*: diploid multigerm, good root

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yield, good sugar yield) obtained from the Sugar Institute (Ankara, Turkey) were first rinsed with tap water and then with sterile distilled water. Later, they were disinfected in 70% EtOH for 5 minutes, rinsed with sterile distilled water, and then soaked in different concentrations (Table 1) of a commercial bleach (Domestos) containing 5% (v/v) sodium hypochlorite with the addition of Tween 20 (0.5 ml per 100 ml) for 30 minutes. 30 seeds were used for each line and repeated three times (i.e., a total of 90 seeds for each line). The seeds were then rinsed with and left in sterile distilled water for 18 hours in an incubator at 25 °C. Lastly, they were rinsed with various concentrations (Table 1) of a biocide solution (PPM™, Plant Preservative Mixture, Plant Cell Technology Inc., WA, USA) before being placed on a medium for germination. The germination medium was solid MS basal medium (Murashige and Skoog, 1962) containing 1, 3 or 5 mg/L TDZ (thidiazuron) supplemented with 3% (w/v) sucrose and 0.8% (w/v) agar (Oxoid No.3). The seeds were incubated in an incubator at 25±1 °C, 12 h light/12 h dark regime for five weeks. Petiole explants were excised from these pretreated axenic seedlings of each breeding line and cultured on two different regeneration media; i) the RGL medium contained 0.5 mg/L BAP and 0.1 mg/L NAA and ii) the RGH medium contained 1 mg/L BAP and 0.3 mg/L NAA. Incubation conditions were the same as the germination medium. When adventitious shoots developing from the cultured petiole explants reached to 3-4 cm long, they were transferred to a rooting medium containing solid MS medium supplemented with 1 or 3 mg/L NAA. Experiments were repeated three times each using 10 explants per treatment. The number of petiole explants giving rise to shoots were recorded and expressed as the mean number of shoots per explant. To compare the treatment means, the data were subjected to standard analysis of variance and Duncan's Multiple Range Test.

RESULTS and DISCUSSION

Surface Sterilization and Germination of Seeds. Surface sterilization of sugar beet seeds is often a problem because of the rough surface of the seeds. The use of the previously published protocols (Tetu et al., 1987; Freytag et al., 1988; Ritchie et al., 1989; Zhong et al., 1993) failed to produce successful sterilization with our material since the treatments that were effective in removing contaminants were also toxic to the plant material. Therefore, we first developed an effective seed sterilization method using various concentrations of Domestos and PPM™ in different combinations (Table 1). When 50% Domestos was used without a further treatment with PPM™, a 66% of seed sterilization rate was achieved but none of the sterilized seeds was able to germinate. Apparently, such a high concentration of Domestos containing NaOCl as an active ingredient was

Table 1. Effects of sequential treatments of sugar beet seeds with different concentrations and combinations of Domestos (DOM, submerged for 30 mins) and PPM™ (rinsed for few seconds) on % seed sterilization rate and % of seeds germinated on MS basal medium. 30 seeds were used for each line and repeated three times (i.e., a total of 90 seeds for each line).

Concentration (%) of DOM/PPM™	Percent Seed Sterilization Achieved	Percent Seed Germinated
	(mean of three lines)	(mean of three lines)
50/0	66	0
25/0	65	8.3
5/0	65	9.0
5/50	100	21.6
2.5/25	100	31.6
2.5/10	100	43.6
1/10	100	62.0
0.5/25	100	62.0
0.5/10	100	67.0
0.5/5	100	75.6

toxic to the embryo. When Domestos concentration was decreased to 25% or 5% without a subsequent PPM™ treatment, the rate of seed sterilization remained almost constant (65% compared to 66%) but a small proportion of the seeds could germinate (8.3% and 9%, respectively). As shown in Table 1, seed sterilization rate readily reached to 100% when a further treatment with varying concentrations of PPM™ in combination with lower concentrations of Domestos was employed although the combination of relatively high (5%) Domestos and the highest concentration (50%) of PPM™ resulted in relatively lower germination rates (21.6%). We also demonstrated that at a given Domestos concentration, percentages of seeds germinated steadily increased with decreasing PPM™ concentrations, reaching to 75.6% at the combination of 0.5% Domestos and 5% PPM™. This suggested that although, PPM™ had a more significant role in eradicating the surface contaminants than Domestos, it was not sufficient to achieve a 100% sterilization rate when used alone at concentrations of 10% and 25% (data not shown).

PPM™ containing two isothiazolone biocides is a relatively new, broad-spectrum agent developed by Plant Cell Technology Inc. (USA) and has been increasingly used in tissue cultures of many species including salad burnet, melon, petunia, tobacco, chrysanthemum, European birch and rhododendron at concentrations ranging from 0.5 to 10 ml/L (Babaoglu and Yorgancilar, 2000; Crompton and Koch, 2001; George and Tripepi, 2001). It is either used for surface sterilization or included in the culture medium to eliminate the internal contaminants that may be present in explants. Babaoglu and Yorgancilar (2000) reported that the use of PPM™ was effective in controlling contamination without

Table 2. Analysis of variance of shoot production from petiole explants excised from TDZ-pretreated seedlings of three sugar beet breeding lines grown on MS medium containing 1, 3 or 5 mg/L BAP for five weeks and then cultured on two regeneration media containing either 0.5 mg/L BAP and 0.1 mg/L NAA (RGL medium) or 1 mg/L BAP and 0.3 mg/L NAA (RGH medium).

Source of Variation	D.F.	Mean Square
Replication	2	0.009
Lines (A)	2	1.743 **
Germination Media (B)	2	0.318 **
Regeneration Media (C)	1	4.122 **
A X B	4	0.795 **
A X C	2	0.325 **
B X C	2	0.048 NS
A X B x C	4	0.363 **
Error	34	0.036

** P = 0.01 and 0.05, NS: non-significant

impairing the shoot regeneration from petiole and hypocotyl explants of salad burnet (*Poterium sanguisorba* L.). Our results are consistent with their findings that PPM™ increased the rate of seed sterilization while being gentle to the embryo.

We have previously encountered serious problems with surface sterilization of various sugar beet explants including ovules, seeds, petioles, hypocotyls and leaves of greenhouse- or field-grown plants when tested various sterilization agents, mostly those containing hypochlorites (Gürel et al., unpublished). In addition, the published protocols (Tetu et al., 1987; Freytag et al., 1988; Ritchie et al., 1989; Zhong et al., 1993) that we followed produced inconsistent results. Most of the previous protocols used a wide range of agents combined with various sequential treatments, which made the sterilization procedure more labourous and complex, but the rates of sterility achieved ranged from as low as 5% to 90%. The seed sterilization procedure we developed here is much more successful than the previously published procedures as a high rate of seed sterilization was achieved without reducing the germinability of sugar beet seeds. To our knowledge, this is the first study reporting the use of

PPM™ in sugar beet tissue culture. In case PPM™ is used for surface sterilization of sugar beet explants, we suggest the use of a wide range of concentrations when using it for the first time on the untested materials. Thus, Crompton and Koch (2001) tested the effects of PPM™ on adventitious shoot regeneration in petunia, somatic embryogenesis in melon and androgenesis in tobacco, and reported that the effectiveness of PPM™ was largely dependent on the plant species tested.

Effects of TDZ Pretreatment on Direct Shoot Development. The effect of different TDZ levels used in the germination medium on subsequent direct shoot development from petiole explants of sterile seedlings of three sugar beet (*Beta vulgaris* L.) breeding lines was examined. Petiole explants were excised from the seedlings that were grown on medium containing 1, 3 or 5 mg/L TDZ for five weeks and then placed on two different regeneration media containing a low or a high combination of BAP and NAA; either 0.5 mg/L BAP and 0.1 mg/L NAA (i.e., RGL medium) or 1 mg/L BAP and 0.3 mg/L NAA (i.e., RGH medium). Results of analysis of variance of the shoot production data are presented in Table 2.

When different TDZ levels of the germination medium were compared regardless of the other parameters, it was found that 1 mg/L TDZ was significantly more effective than either 3 or 5 mg/L TDZ, producing a mean of 1.67 shoots per explant compared to 1.16 or 0.92 shoots per explant at 3 or 5 mg/L TDZ, respectively (Table 3). However, explants taken from seedlings germinated on hormone-free medium produced no shoots at all (data not provided), but little callus only, when subsequently cultured on either RGL or RGH medium, as also reported by Zhong et al. (1993) and Zhang et al. (2001). In terms of the type of the regeneration medium, RGL medium was always superior to the RGH medium for every single line, mean number of shoots per explant being 1.26 compared to 0.86 for the line M114, 1.94 compared to 1.28 for the line ELK345 and 1.43 compared to 0.75 for the line M1017. The promotive effect of the RGL medium on regeneration frequency is more pronounced in Table 4 in which the means of two

Table 3. An overall comparison of different lines, germination media containing 1, 3 or 5 mg/L TDZ and regeneration media containing either 0.5 mg/L BAP and 0.1 mg/L NAA (RGL medium) or 1 mg/L BAP and 0.3 mg/L NAA (RGH medium) in terms of the mean number of shoots per explant. Means with the same letter within rows and columns are not significantly different at both $p=0.01$ and $p=0.05$. The data of this table is the same as that of Table 4 but presented in a different way.

Germination Medium	Mean Number of Shoots per Explant						Mean
	Line M114		Line ELK345		Line M1017		
	RGL	RGH	RGL	RGH	RGL	RGH	
1 mg/L TDZ	1.76	1.30	2.87	1.67	1.47	0.97	1.67 ^a
3 mg/L TDZ	0.84	0.73	1.84	1.23	1.60	0.73	1.16 ^b
5 mg/L TDZ	1.17	0.54	1.10	0.94	1.23	0.56	0.92 ^b
Mean	1.26 ^b	0.86 ^c	1.94 ^a	1.28 ^b	1.43 ^b	0.75 ^c	

Table 4. An overall comparison of different lines, germination media containing 1, 3 or 5 mg/L TDZ and regeneration media containing either 0.5 mg/L BAP and 0.1 mg/L NAA (RGL medium) or 1 mg/L BAP and 0.3 mg/L NAA (RGH medium) in terms of the mean number of shoots per explant. Means with the same letter within rows and columns are not significantly different at both $p=0.01$ and $p=0.05$. The data of this table is the same as that of Table 3 but presented in a different way.

	Mean Number of Shoots per Explant									Means
	Line M114			Line ELK345			Line M1017			
	1 TDZ	3 TDZ	5 TDZ	1 TDZ	3 TDZ	5 TDZ	1 TDZ	3 TDZ	5 TDZ	
RGL Medium (mg/L) (0.5 BAP + 0.1 NAA)	1.76	0.84	1.17	2.87	1.84	1.10	1.47	1.60	1.23	1.54^a
RGH Medium (mg/L) (1.0 BAP + 0.3 NAA)	1.30	0.73	0.54	1.67	1.23	0.94	0.97	0.73	0.56	0.96^b
Means of BAP Levels	1.53^b	0.79^c	0.86^{de}	2.27^a	1.54^b	1.02^c	1.22^{bc}	1.17^{bc}	0.90^d	
Means of Lines	1.06^b			1.61^a			1.10^b			

Table 5. An overall comparison of lines and two different rooting media containing 1 or 3 mg/L NAA in terms of the number and mean percentage of rooted shoots. Shoots were obtained from petiole explants excised from sterile seedlings grown on solid MS medium containing 1, 3 or 5 mg/L TDZ for five weeks. 90 shoots were used for each treatment without taking into consideration from which germination or regeneration medium they were derived.

	Rooting Medium Containing:					
	1 mg/L NAA			3 mg/L NAA		
	M114	ELK345	M1017	M114	ELK345	M1017
Number of rooted shoots	19	22	11	34	58	23
% of rooted shoots	21.1	24.5	12.2	37.8	64.5	25.6
Means of NAA levels (%)	19.3			42.6		

regeneration media were compared regardless of the other parameters. The RGL medium containing a lower combination of BAP and NAA produced nearly 50% more shoots from petiole explants than the RGH medium (1.54 shoots per explant compared to 0.96, respectively). It appears that the combination of higher concentrations of BAP and NAA suppressed shoot development.

The effects of pretreating leaf explants or preconditioning seedlings on the subsequent shoot regeneration were previously examined in sugar beet (Zhong et al., 1993; Krens et al., 1996; Zhang et al., 2001) and other species (Cao et al., 2002; Nhut et al., 2002). Zhong et al. (1993) reported that petiole explants taken from donor plants of two sugar beet cultivars pretreated with 0.5 mg/L BAP were the most prolific in regenerating shoots (23.3% of the explants producing shoots) whereas those pretreated with kinetin produced very few adventitious shoots or those explants excised from non-pretreated seedlings (i.e., seeds were germinated on hormone-free medium) produced no shoots at all. It was also shown, in a recent study (Zhang et al., 2001), that no shoots developed from petiole or lamina explants of sugar beet seedlings that were precultured on hormone-free medium when the explants were subsequently

cultured on a regeneration medium containing 1, 2 or 4 mg/L BAP. These results are consistent with our findings which suggest that the capacity for shoot regeneration is predetermined at an early stage of leaf differentiation and, therefore, TDZ is required for maintaining the regenerative capacity. In contrast to our findings and the other previous results (Zhong et al., 1993; Zhaong et al., 2001), Krens et al. (1996) reported that the inclusion of 1 mg/L BAP, TDZ or 2ip in the seed germination medium had no significant effect on the subsequent shoot regeneration capacities of cotyledonary explants when cultured on medium containing 0.22 mg/L BAP. They found that 66.7% of the explants excised from non-pretreated seedlings produced shoots while 69.4%, 77.8% and 73.6% of the explants taken from seedlings pretreated with 1 mg/L BAP, TDZ or 2ip, respectively, were able to produce shoots. This may be attributed to the phenomena of genotypic variation since they used only one accession instead of a range of materials.

When different levels of TDZ of the germination medium were compared for each line separately (see the second row from the bottom in Table 4), their behaviours were consistent with the overall means presented in the last column in Table 3, explants of all lines producing significantly

more shoots when pretreated with 1 mg/L TDZ than with 3 or 5 mg/L TDZ. However, there was a significant variation among the lines when the means of each line was statistically compared regardless of the other parameters (Table 4). Line ELK345 was more productive with a mean of 1.61 than both line M114 (1.06) and line M1017 (1.10). Sugar beet is a heterozygous species and genotypic variation can be a serious problem in experimental work. Previous studies indicate that some genotypes of sugar beet are more amenable to tissue culture than others (Jacq et al., 1992; Zhong et al., 1993; Gürel, 1997; Saunders and Tsai, 1999; Gürel et al., 2000). Even a significant inter-plant (intra-genotype) and inter-leaf (intra-plant) variability was also reported to exist within a sugar beet cultivar, cv. Primo (Gürel, 1997). There are several possible ways of coping with problems of variability in experimental material. One might be to use large numbers of replicates, as suggested by Doley and Saunders (1989), but the intensive nature of plant tissue cultures and the need to standardize the production of the parent plants often restricts the feasibility of this approach. An alternative method might be to screen out material with low organogenic/embryogenic potential and to use only those, which regenerate readily *in vitro*. As genotypes and different regimes of plant growth regulator treatments have significant effects on plant regeneration in sugar beet, testing further combinations of pretreatment and regeneration protocols may also be worthwhile since a rapid and reproducible regeneration system is of extreme significance for the development of a routine transformation procedure for sugar beet.

Effects of NAA Concentration on Rooting of the Regenerated Shoots.

The regenerated shoots were transferred to two different rooting media containing MS salts supplemented with either 1 or 3 mg/L NAA. A total of 90 shoots were used for each treatment without taking into consideration from which germination or regeneration medium they were obtained. As shown in Table 5, 3 mg/L NAA was more effective for root induction than 1 mg/L NAA in all lines. 42.6% of the shoots cultured on a medium containing 3 mg/L NAA produced roots whereas the rooting ratio was 19.3% when the shoots were cultured on a medium containing 1 mg/L NAA. Gürel and Wren (1995) previously demonstrated the significance of NAA for adventitious root development from petiole explants of sugar beet cv. Primo and reported that a minimum of 24 hour exposure to high NAA was necessary for root induction, however, prolonged exposure inhibited root differentiation. As reported previously (Gürel, 1997), genotypic variation was again evident in terms of rooting. The line ELK345 was again the most successful, nearly half of the shoots (i.e. 80 shoots which represents 44.5%) producing roots while 53 (29.5%) and 34 (18.9%) shoots rooted in the lines M114 and M1017, respectively.

In conclusion, it appears that preconditioning the source material of the explants has a significant influence on the

capacity of subsequent *in vitro* regeneration in many plant species, as well as in sugar beet. This effect is more important for recalcitrant species like sugar beet and further refinement of the treatments may result in better protocols of regeneration for valuable sugar beet materials.

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REFERENCES

- Atanassov, A.I. 1986. Sugar beet. *In Handbook of Plant Cell Culture*, ed. D.A. Evans, W.R. Sharp and P.V. Ammirato, pp 652-680, New York: MacMillan.
- Babaoglu, M. and Yorgancilar, M. 2000. TDZ-specific plant regeneration in salad burnet. *Plant Cell Tissue and Organ Culture* 63: 31-34.
- Cai, D.G., Kleine, M., Kifle, S., Harloff, H.J., Sandal, N.N., Marcker, K.A., KleinLankhorst, R.M., Salentijn, E.M.J., Lange, W., Stiekema, W.J., Wyss, U., Grundler, F.M.W. and Jung, C. 1997. Positional cloning of a gene for nematode resistance in sugar beet. *Science* 275: 832-834.
- Cao, X.L., Hammerschlag, F.A. and Douglass, L. 2002. A two-step pretreatment significantly enhances shoot organogenesis from leaf explants of highbush blueberry cv. Bluecrop. *Hortscience* 37: 819-821.
- Crompton, M.E. and Koch, J.M. 2001. Influence of plant preservative mixture (PPMTM) on adventitious organogenesis in melon, petunia and tobacco. *In Vitro Cellular and Developmental Biology - Plant* 37: 259-261.
- Detrez, C., Tetu, T., Sangwan, R.S., Sangwan-Norreel, B.S. 1988. Direct organogenesis from petiole and thin cell layer explants in sugar beet cultured *in vitro*. *Journal of Experimental Botany* 39: 917-926.
- D'Halluin, K., Bossout, M., Bonne, M., Mazur, B., Leemans, J. and Botterman, J. 1992. Transformation of sugarbeet (*Beta vulgaris* L.) and evaluation of herbicide resistance in transgenic plants. *Biotechnology* 10: 309-314.
- Doley, W.P. and Saunders, J.W. 1989. Hormone-free medium will support callus production and subsequent shoot regeneration from whole plant leaf explants in some sugar beet (*Beta vulgaris* L.) populations. *Plant Cell Reports* 8: 222-225.

- Elliott, M.C., Chen, D.F., Fowler, M.R., Kirkby, M.J., Kubalaková, M., Scott, N.W., Zhang, C.L. and Slater, A. 1996. Towards the perfect sugar beet via gene manipulation. *Sugar Crops China* 1: 23-30.
- Freytag, A.H., Anand, S.C., Rao-Arelli, A.P. and Owens, L.D. 1988. An improved medium for adventitious shoot formation and callus induction in *Beta vulgaris* L. *in vitro*. *Plant Cell Reports* 7: 30-34.
- Ivic, S.D., Sicher, R.C. and Smigocki, A.C. 2001. Growth habit and sugar accumulation in sugarbeet (*Beta vulgaris* L.) transformed with a cytokinin biosynthesis gene. *Plant Cell Reports* 20: 770-773.
- George, M.W. and Tripepi, R.R. 2001. Plant Preservative Mixture™ can affect shoot regeneration from leaf explants of chrysanthemum, European birch and rhododendron. *Hortscience* 36: 768-769.
- Grieve, T.M., Gartland, K.M.A. and Elliott, M.C. 1997. Micropropagation of commercially important sugar beet cultivars. *Plant Growth Regulation* 21: 15-18.
- Gürel, E. and Wren, M.J. 1995. *In vitro* development from leaf explants of sugar beet (*Beta vulgaris* L.): Rhizogenesis and the effect of sequential exposure to auxin and cytokinin. *Annals of Botany* 75: 31-38.
- Gürel, E. 1997. Callus and root development from leaf explants of sugar beet (*Beta vulgaris* L.): Variability at variety, plant and organ level. *Turkish Journal of Botany* 21: 131-136.
- Gürel, S., Gürel, E. and Kaya, Z. 2000. Doubled haploid plant production from unpollinated ovules of sugar beet (*Beta vulgaris* L.). *Plant Cell Reports* 19: 1155-1159.
- Jacq, B., Tetu, T., Sangwan, R.S., Laats, A.D. and Sangwan-Norreel, B.S. 1992. Plant regeneration from sugar beet (*Beta vulgaris* L.) hypocotyls cultured *in vitro* and flow cytometric nuclear DNA analysis of regenerants. *Plant Cell Reports* 11: 329-333.
- Krens, F.A., Trifonova, A., Keizer, L.C.P. and Hall, R.D. 1996. The effect of exogenously-applied phytohormones on gene transfer efficiency in sugar beet (*Beta vulgaris* L.). *Plant Science* 116: 97-106.
- Mannerlof, M., Tuvešson, S., Steen, P. and Tenning, P. 1997. Transgenic sugar beet tolerant to glyphosate. *Euphytica* 94: 83-91.
- Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* 15: 473-497.
- Nhut, D.T., Huong, N.T.D., Van, L.B., DaSilva, J.T., Fukai, S. and Tanaka, M. 2002. The changes in shoot regeneration potential of protocorm-like bodies derived from *Lilium longiflorum* young stem explants exposed to medium volume, pH, light intensity and sucrose concentration pretreatment. *Journal of Horticultural Science and Biotechnology* 77: 79-82.
- Ritchie, G.A., Short, K.C., Davey, M.R. 1989. *In vitro* shoot regeneration from callus, leaf axils and petioles of sugar beet (*Beta vulgaris* L.). *Journal of Experimental Botany* 40: 277-283.
- Saunders, J.W. and Tsai, C.J. 1999. Production of somatic embryos and shoots from sugar beet callus: Effects of abscisic acid, other growth regulators, nitrogen source, sucrose concentration and genotype. *In Vitro Cellular and Developmental Biology - Plant* 35: 18-24.
- Tetu, T., Sangwan, R.S. and Sangwan-Norreel, B.S. 1987. Hormonal control of organogenesis and somatic embryogenesis in *Beta vulgaris* callus. *Journal of Experimental Botany* 38: 506-517.
- Trifonova, A. and Atanasov, A. 1995. Genetic transformation of sugar beet by *Agrobacterium rhizogenes*. *Biotechnology and Biotechnological Equipment* 9: 23-26.
- Zhang, C.L., Chen, D.F. and Elliott, M.C. 2001. Thidiazuron-induced organogenesis and somatic embryogenesis in sugar beet (*Beta vulgaris* L.). *In Vitro Cellular and Developmental Biology - Plant* 37: 305-310.
- Zhong, Z., Smith, H.G. and Thomas, T.H. 1993. *In vitro* culture of petioles and intact leaves of sugar beet (*Beta vulgaris* L.). *Plant Growth Regulation* 12: 59-66.