Plant Cell Tiss Organ Cult (2011) 106:261–268
DOI 10.1007/s11240-011-9916-8

ORIGINAL PAPER

A two-stage pretreatment of seedlings improves adventitious shoot regeneration in sugar beet (*Beta vulgaris* L.)

Songul Gurel · Mehmet Cengiz Baloglu · Ekrem Gurel · Huseyin Avni Oktem · Meral Yucel

Received: 23 October 2010 / Accepted: 4 January 2011 / Published online: 14 January 2011

Abstract The effects of a two-stage pretreatment of seedlings on the subsequent shoot regeneration capacity were investigated. Pretreated seedlings were obtained by germinating seeds on three different *germination* media and then further culturing on six different *growth* media. Lamina and petiole explants of two sugar beet (*Beta vulgaris* L.) breeding lines were then excised from the pretreated seedlings and cultured on five different shoot *regeneration* media. In both breeding lines, petiole explants produced significantly more shoots than lamina explants with higher frequencies of organogenic capacities; petiole explants of the lines M1195 and ELK345 produced a mean of 2.1 and 2.7 shoots per explant while their lamina explants produced 1.5 and 2.2 shoots per explant, respectively. A genotypic variation was evident as the line ELK345 was more productive for shoot development from both types of explants. In overall comparisons of different *germination*, *growth* and *regeneration* media, germination medium was most effective when supplemented with 0.5 mg/l 6-benzyladenine (BA) while both growth and regeneration media were most productive when contained a combination of 0.25 mg/l BA and 0.10 mg/l indole-3-butyric acid (IBA). Of all the treatments tested, the highest mean number of shoots per explant (8.3 shoots) and frequency of organogenic explants (75.6%) were obtained on regeneration medium supplemented with 0.25 mg/l BA and 0.10 mg/l IBA when petiole explants of the line ELK345 were excised from the seedlings that had been germinated on medium containing 0.5 mg/l BA followed by further growth on medium containing 0.25 mg/l BA and 0.10 mg/l IBA.

Keywords Sugar beet · *Beta vulgaris* L. · Two-stage pretreatment · Shoot regeneration · Petiole explants · Lamina explants

S. Gurel (✉)
Department of Plant Breeding, Sugar Institute, Etimesgut, 06790 Ankara, Turkey
e-mail: songul_gurel@yahoo.com

M. C. Baloglu · H. A. Oktem · M. Yucel
Department of Biological Sciences, Middle East Technical University, 06531 Ankara, Turkey

E. Gurel
Department of Biology, Abant Izzet Baysal University, 14280 Bolu, Turkey

Introduction

Sugar beet (*Beta vulgaris* L.) is the most important cultivated plants used for sugar production in Europe and one of only two plant sources from which sucrose (i.e., sugar) can be economically produced in the world. Sugar cane and sugar beet contribute to the total sugar production by 80 and 20%, respectively (Turkish Sugar Co. 2010). Sugar beet is known to be a recalcitrant species with respect to in vitro culture. Also, a high degree of genotypic variation, primarily because of its highly heterozygous nature due to outcrossing, is a serious problem for the optimization of both regeneration (Gurel 1997; Gurel et al. 2001; Zhang et al. 2008) and transformation protocols in sugar beet (Krens et al. 1996; Zakharchenko et al. 2000; Hisano et al. 2004; Ninkovic et al. 2010) as well as in many other species (Arellano et al. 2009; Watt et al. 2009; Liu et al. 2010; Kwapata et al. 2010). The effects of pretreating leaf explants or preconditioning the source of explants (seedlings) on the subsequent regeneration efficiency were previously reported for sugar beet (Jacq et al. 1993; Zhong

et al. 1993a, b; Zhang et al. 2001; Gurel et al. 2003a) and several other species (D'Onofrio and Morini 2006; Thomas 2007). It was shown that preconditioning sugar beet seedlings on medium containing 6-benzylaminopurine (BA) (Jacq et al. 1993; Zhong et al. 1993a, b; Zhang et al. 2001; Gurel et al. 2003a), thidiazuron (TDZ) (Zhang et al. 2001; Gurel et al. 2003b) or 2,3,5-triiodobenzoic acid (TIBA) alone or combined with either BA (Jacq et al. 1993) or L-proline (Moghaddam et al. 2000) improved regeneration efficiency from different types of explants. Our previous studies tested a single-stage pretreatment of three sugar beet breeding lines by germinating seedlings on medium containing different concentrations of BA (Gurel et al. 2003a) or TDZ (Gurel et al. 2003b) and then culturing excised petiole explants of pretreated seedlings on two different regeneration media. In this study, the effects of a two-stage pretreatment on adventitious shoot regeneration from tissues of sugar beet (*Beta vulgaris* L.) was investigated.

Materials and methods

Pretreatment of seedlings and shoot regeneration

Two sugar beet breeding lines (M1195 and ELK345, both being monogerm, and having high root yield and sugar content) developed at Sugar Institute (Ankara, Turkey) were used. The seeds were surface sterilized in 70% ethanol for 5 min, then kept in 7.5% sodium hypochlorite solution for 1 h by continuous stirring with magnetic stirrer and finally rinsed three times with sterile distilled water. Seeds were kept in sterile distilled water overnight in dark at 25°C for imbibition, then rinsed in 5% PPM^{TM} solution (Plant Preservation Mixture, Plant Cell Technology Inc. WA, USA) for 10 min. The sterilised seeds were germinated on three different *germination* media which contained Murashige and Skoog (MS) medium (Murashige and Skoog 1962) supplemented with no plant growth regulators (PGRs, control), 0.5 mg/l TIBA (an anti-auxin) or 0.5 mg/l BA and kept on these media for 4 weeks (*first* pretreatment) (see Fig. 1 for experimental layout). The germinated seedlings were then further cultured on six different *growth* media containing MS medium supplemented with no PGRs, 0.5, 1.0, 3.0 mg/l BA, 0.5 mg/l TIBA or a combination of 0.25 mg/l BA and 0.10 mg/l indole-3-butyric acid (IBA) for 4 weeks (*second* pretreatment). Finally, leaf lamina pieces (5 mm × 5 mm) and petiole segments (5 mm) were excised from the pretreated seedlings, and cultured on five different shoot *regeneration* media, which contained MS medium supplemented with no PGRs, 1.0, 3.0 mg/l BA, 0.5 mg/l TIBA or a combination of 0.25 mg/l BA and 0.10 mg/l IBA for 6 weeks (Fig. 1). All the culture media used throughout this study, including rooting experiments, were supplemented with 2.2 mg/l MS basal powder, 3% sucrose and 0.8% agar, pH was adjusted to 5.8 with 0.1 N HCl or 0.1 N KOH before autoclaving, and the cultures were kept at 24 ± 2°C under a 16/8 h (light/dark) photoperiod provided by cool-white fluorescent light with 50 $\mu mol^{-2}s^{-1}$ irradiance.

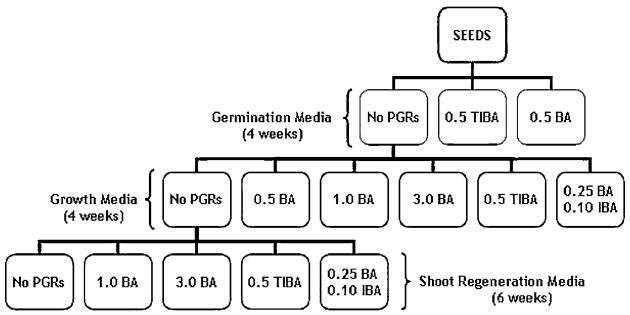

Fig. 1 Experimental layout showing a two-stage hormonal pretreatment by germinating seeds on three different *germination* media for 4 weeks and then further growing on six different *growth* media before culturing the explants excised from the pretreated seedlings on five different *regeneration* media for 6 weeks

Rooting and hardening off

Regenerated shoots (8–10 cm long) were rooted on MS medium containing 1.0 mg/l IBA. After 4 weeks of culture on rooting medium, the rooted plantlets were washed with tap water and then transfered to pots containing compost. For acclimatization of the regenerants, pots were covered with transparent plastic bags to avoid desiccation. The plastic bags were gradually removed in 2 weeks and plants were maintained in the greenhouse for 4 weeks before being transferred to the field. A high rate of acclimatization was achieved; 97% of the plantlets were able to survive through greenhouse and field conditions.

Data collection and statistical analysis

Each treatment used 15 explants and the experiments were repeated in triplicate, thus using a total of 45 replicates per treatment. The mean number of shoots per explant and the mean frequencies (%) of organogenic explants were determined after a 6 weeks of culture on regeneration media. The data were statistically analyzed by using Microsoft Excel (Office 2007, ToolPak Analyser). Analysis of variance was used to test the statistical significance and, using an internet-based free program, the significance of differences among treatment means was done manually according to Tukey's test at $P = 0.05$.

Results

The effects of a two-stage hormonal pretreatment of seedlings on shoot regeneration capacity were investigated using two different types of explants isolated from two sugar beet breeding lines. A substantial amount of data were obtained by testing a total of 360 different treatments and generating two different types of data; mean number of shoots per explant and mean frequency of organogenic explants. Comparisons of different types of explants and breeding lines (Fig. 2) and different medium compositions used during *germination* (Fig. 3), *growth* (Fig. 4) and *regeneration* stages (Fig. 5) were made irrespective of the other parameters tested.

Comparison of explant types and breeding lines

In both breeding lines, petiole explants developed significantly more shoots than lamina explants with higher frequencies of organogenic capacities (Fig. 2). Petiole explants of the lines M1195 and ELK345 produced a mean of 2.1 and 2.7 shoots per explant while their lamina explants produced 1.5 and 2.2 shoots per explant, respectively. A very similar pattern was also clear in terms of the mean frequencies of organogenic explants; 29.9 and 44.3% of petiole explants of the lines M1195 and ELK345 were able to produce shoots while 16.0 and 22.9% of the lamina explants produced shoots, respectively. It was also clear that the differences between two breeding lines in terms of the mean number of shoots per explant from both types of explants (petiole and lamina) were statistically significant. However, while the differences in terms of the mean frequencies of organogenic explants were significant for petiole explants, they were not significant for lamina explants (Fig. 2). Both petiole and lamina explants of the line ELK345 produced significantly more shoots with higher frequencies of organogenic capacity than those of the line M1195; e.g., petiole explants of the line ELK345 produced a mean of 2.7 shoots per explant with 44.3% frequency of organogenic explants while the same type of explants of the line M1195 produced a mean of 2.1 shoots per explant with 29.9% frequency.

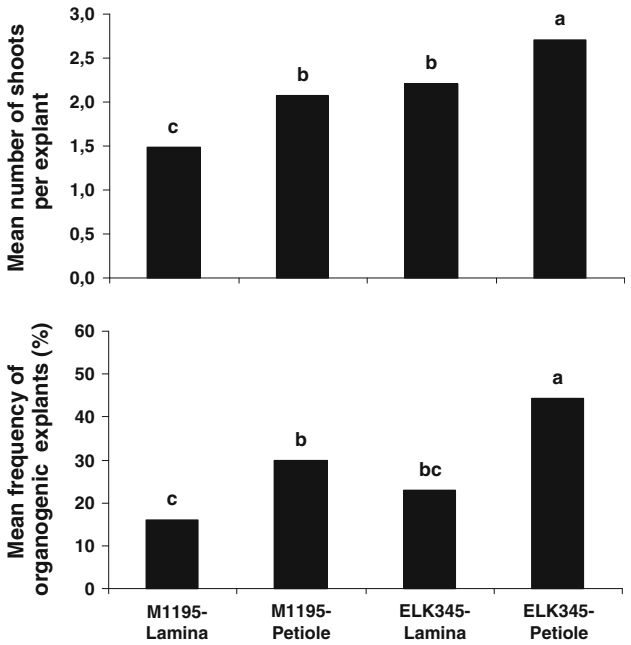

Fig. 2 Comparison of two different *explant types* (lamina and petiole) and *breeding lines* (M1195 and ELK345) for shoot regeneration capacities expressed as the mean number of shoots per explant and mean frequency (%) of organogenic explants. Overall comparisons were made irrespective of the medium composition used for the stages of *germination*, *growth* and *regeneration*. *Letters* above the *bars* indicate the significance of differences between mean values ($P = 0.05$)

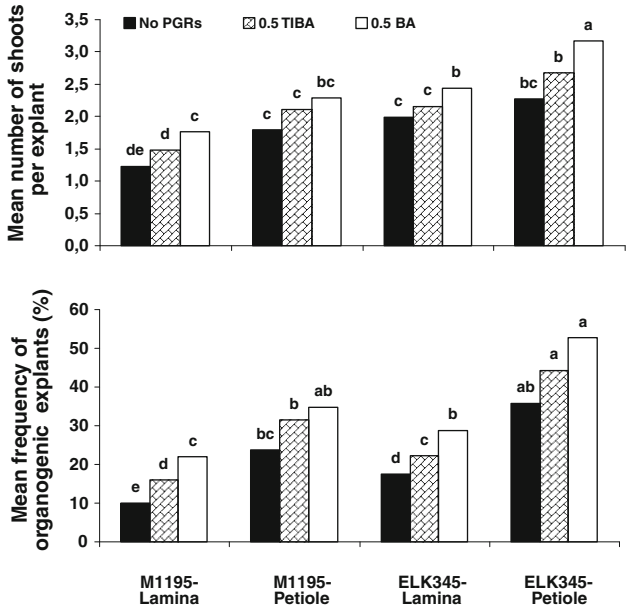

Fig. 3 Comparison of three different *germination* media containing no PGRs, 0.5 mg/l TIBA or 0.5 mg/l BA for shoot regeneration capacities (expressed as the mean number of shoots per explant and mean frequency of organogenic explants) of lamina and petiole explants isolated from two breeding lines (M1195 and ELK345). Overall comparisons were made irrespective of the medium composition used for the stages of *growth* and *regeneration*. *Letters* above the *bars* indicate the significance of differences between mean values ($P = 0.05$)

On the other hand, lamina explants of both breeding lines produced no shoots at all on any of the regeneration media when they were excised from seedlings that had been germinated and grown on hormone-free medium (data not provided). For petiole explants, the line M1195 was able to produce shoots, though at low numbers and frequencies, on all regeneration media while the line ELK345 did not produce any shoots at all when regeneration

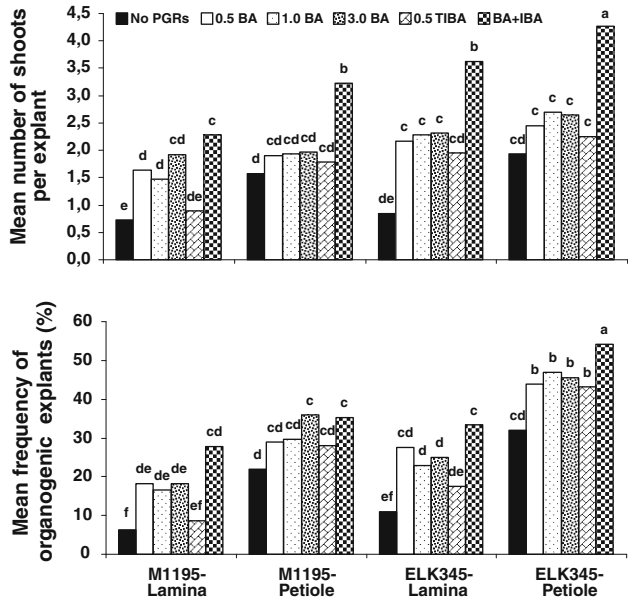

Fig. 4 Comparison of six different *growth* media containing no PGRs, 0.5, 1.0, 3.0 mg/l BA, 0.5 mg/l TIBA or a combination of 0.25 mg/l BA and 0.10 mg/l IBA [BA + IBA] for shoot regeneration capacities (expressed as the mean number of shoots per explant and mean frequency of organogenic explants) of lamina and petiole explants isolated from two breeding lines (M1195 and ELK345). Overall comparisons were made irrespective of the medium composition used for the stages of *germination* and *regeneration*. *Letters* above the *bars* indicate the significance of differences between mean values ($P = 0.05$)

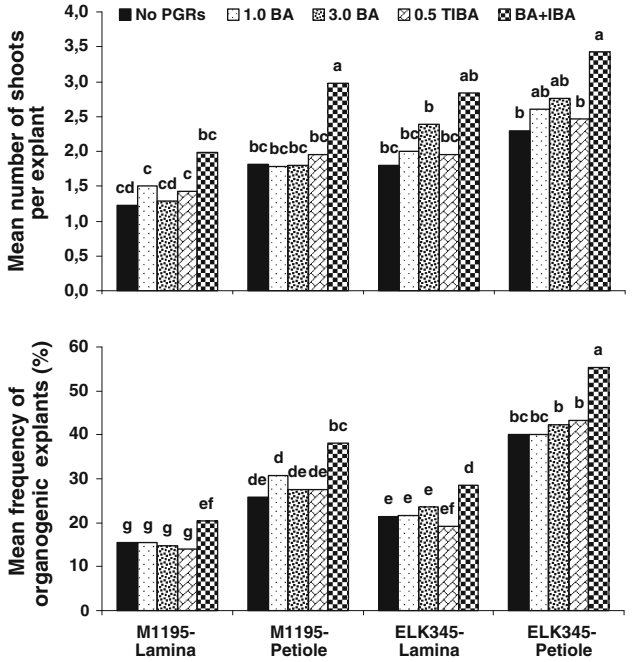

Fig. 5 Comparison of five different *regeneration* media containing no PGRs, 1.0, 3.0 mg/l BA, 0.5 mg/l TIBA or a combination of 0.25 mg/l BA and 0.10 mg/l IBA [BA + IBA] for shoot regeneration capacities (expressed as the mean number of shoots per explant and mean frequency of organogenic explants) of lamina and petiole explants isolated from two breeding lines (M1195 and ELK345). Overall comparisons were made irrespective of the medium composition used for the stages of *germination* and *growth*. *Letters* above the *bars* indicate the significance of differences between mean values ($P = 0.05$)

medium contained no PGRs or 1.0 mg/l BA (data not provided).

Comparison of different germination media

When three different germination media were compared for shoot regeneration capacity, the medium containing no PGRs was found the least effective in both breeding lines and explant types (Fig. 3). When culture medium was supplemented with 0.5 mg/l BA, higher mean numbers of shoots per explant with higher frequencies of organogenic explants were obtained. Petiole explants of the line ELK345 produced the highest mean number of shoots per explant (3.2 shoots) and frequency of shoot organogenesis (52.8%) when cultured on medium containing 0.5 mg/l BA while lamina explants of the line M1195 produced the lowest mean number of shoots per explant (1.2 shoots) and frequency of shoot organogenesis (9.9%) when cultured on hormone-free medium. On the other hand, medium containing 0.5 mg/l TIBA appeared more productive than hormone-free medium but much less effective than the medium containing 0.5 mg/l BA, especially, in terms of the mean number of shoots per explant (Fig. 3).

Comparison of different growth media

When six different growth media were compared for shoot regeneration capacity (Fig. 4), the medium containing no PGRs was again found the least productive in both breeding lines and explant types. In terms of both mean number of shoots per explant and frequency of explants developing shoots, it was observed that increasing concentrations of BA from 0.5 to 3.0 mg/l did not have a distinctive promoting effect. However, the BA-containing media were slightly more productive than the medium containing 0.5 mg/l TIBA. The most productive result was achieved when the medium contained a combination of 0.25 mg/l BA and 0.10 mg/l IBA in both lines and explant types; petiole explants of the line ELK345 producing the highest mean number of shoots per explant (4.3 shoots) and frequency of organogenic explants (54.2%) (Fig. 4).

Comparison of different regeneration media

With respect to the regeneration capacities of two different types of explants excised from two breeding lines and cultured on five different regeneration media, those media

containing no PGRs, 1.0 or 3.0 mg/l BA or 0.5 mg/l TIBA resulted in similar levels of shoot regeneration capacities (Fig. 5). However, in most of the treatments, the medium containing a combination of 0.25 mg/l BA and 0.10 mg/l IBA produced significantly more shoots with higher frequencies of organogenic explants; petiole explants of the line ELK345 producing the highest mean number of shoots per explant (3.4 shoots) and frequency of organogenic explants (55.4%). Of all the treatments, the highest mean number of shoots per explant (8.3 shoots) and frequency of organogenic explants (75.6%) were obtained on regeneration medium which contained a combination of 0.25 mg/l BA and 0.10 mg/l IBA when petiole explants of the line ELK345 were excised from the seedlings that had been germinated on medium containing 0.5 mg/l BA followed by further growth on medium containing a combination of 0.25 mg/l BA and 0.10 mg/l IBA (data not provided).

Discussion

This paper describes the effects of a two-stage (i.e., *first* pretreatment during *germination* and *second* pretreatment during *growth* stage) hormonal pretreatment of explant sources (seedlings) on shoot regeneration capacity using two different types of explants isolated from two sugar beet breeding lines.

A genotypic variation was evident as both types of explants of the line ELK345 produced significantly more shoots with higher frequencies of organogenic explants than those of the line M1195; petiole explants of the line ELK345 producing approximately 30% more shoots (an overall mean of 2.7 vs. 2.1 shoots per explant) and a 50% higher frequency of organogenic explants (an overall mean of 44.3 vs. 29.9%) than the same explant type of the line M1195 (Fig. 2). Genotypic variation, primarily because of its highly heterozygous nature due to outcrossing, is a serious problem for in vitro regeneration of sugar beet as certain genotypes are more amenable than others to organogenesis (Detrez et al. 1988; Zhong et al. 1993b; Gurel et al. 2001; Mishutkina and Gaponenko 2006) and somatic embryogenesis (Saunders and Tsai 1999; Zhang et al. 2008). To use large numbers of explant replicates from many individual plants within a single line/population was suggested as a mean to cope with variability in sugar beet (Doley and Saunders 1989; Ivic-Haymes and Smigocki 2005) but the laboriousness of plant tissue culture, coupled with the need to standardize production of parental plants, often restricts feasibility of this approach. Source material of explants can be sorted prior to culture to eliminate those with low organogenic or embryogenic potential. To facilitate this approach, attempts were made to correlate blackening of leaf lamina discs on culture medium and the inability to produce roots with levels of naturally-occurring phenolics. The latter was measured through polyphenol oxidase (PPO) activity with a highly sensitive, small-scale, reproducible method (Gurel and Wren 1995b). Although a twofold variation in levels of enzyme activity was observed, no relationship was observed between PPO activity and rooting capacity, or between PPO activity and degree of blackening; only a weak negative correlation was evident between degree of blackening and rooting capacity (Gurel 1997). Other factors, e.g., nature and concentration of phenolic substrates rather than enzyme activity, might be more critical in determining regenerative capacity of sugar beet materials (Gurel et al. 2008).

A significant difference was also evident between the types of explants in both breeding lines; petiole explants of the lines ELK345 and M1195 producing approximately 25 and 35% more shoots than the lamina explants with an overall mean of 2.7 versus 2.2 and 2.1 versus 1.5 shoots per explant, respectively (Fig. 2). The differences were more prominent when the overall mean frequencies of organogenic explants were compared; 44.3 versus 22.9% for petiole and lamina explants of the line ELK345 and 29.9 versus 16.0% for the line M1195, respectively. The type of explant has been shown to be important for in vitro regeneration of several species (Abogadallah and Quick 2010; Cogbill et al. 2010; Li et al. 2010). As also observed in this study, usually petiole explants are more responsive than leaf lamina or other seedling parts in producing adventitious roots (Gurel and Wren 1995a) and shoots (Tetu et al. 1987; Ritchie et al. 1989; Grieve et al. 1997; Zhang et al. 2001; Mishutkina and Gaponenko 2006). This might be attributed to the nature of the rich vascular tissue of the petiole since, at least for direct root organogenesis in sugar beet (Gurel and Wren 1995a), primordial cells mostly develop from parenchyma cells in the vascular cambium along the vascular bundles of the petiole. In contrast, petiole sectioning showed that direct adventitious shoots originated from subepidermal parenchyma cells (Detrez et al. 1988), perhaps explaining why thin cell layer explants were successfully employed for direct (Detrez et al. 1989) and indirect (Toldi et al. 1996) shoot regeneration in sugar beet.

Previous studies in sugar beet have shown that preconditioning seedlings (source of explants) on medium containing BA (Jacq et al. 1993; Zhang et al. 2001; Gurel et al. 2003a), TDZ (Zhang et al. 2001; Gurel et al. 2003b) or TIBA alone or combined with either BA (Jacq et al. 1993) or L-proline (Moghaddam et al. 2000) improved regeneration efficiency from different explants. When Jacq et al. (1993) tested several preconditioning media on organogenic callus induction, pretreatment with BA or TIBA alone was insufficient but highly productive when used in combination. TIBA is known to inhibit polar transport of auxins in plants, which leads to loss of apical dominance and promotion of axillary shoot growth (Liu et al. 1993).

However, it is not known whether effects of TIBA are due to its hormone-like activity, which induces cell division and/or differentiation, or to its effects on the relative concentrations of endogenous auxin in excised tissues.

Our previous studies tested a single-stage pretreatment of three sugar beet breeding lines by germinating seedlings on medium containing different concentrations (1.0, 3.0 or 5.0 mg/l) of BA (Gurel et al. 2003a) or TDZ (Gurel et al. 2003b) for 5 weeks and then culturing excised petiole explants on two different regeneration media containing 0.5 or 1.0 mg/l BA combined with 0.1 or 0.3 mg/l NAA, respectively. The present study, however, examined the effects of a two-stage pretreatment of donor plant materials on the subsequent shoot regeneration capacities of different explants of two breeding lines.

It was observed that the explants isolated from untreated seedlings (i.e., germinated and further grown on medium containing no PGRs) produced either no shoots at all (as in lamina explants) or in very few numbers with low frequencies of organogenic explants (as in petiole explants) (data not provided). This observation was more evident when an overall comparison of different *germination* media containing either no PGRs, 0.5 mg/l BA or TIBA was made irrespective of the hormonal compositions of *growth* and *regeneration* media; both types of explant and breeding lines produced significantly less numbers of shoots per explant with lower frequencies of organogenic explants when cultured on regeneration medium containing either no PGRs or 0.5 mg/l TIBA (Fig. 3). However, those germination media supplemented with 0.5 mg/l BA were much more productive for shoot development than the media containing 0.5 mg/l TIBA. These results were consistent with the findings of Zhong et al. (1993a, b) who reported that petiole explants taken from donor plants of two sugar beet cultivars pretreated with 0.5 mg/l BA were the most prolific in regenerating shoots whereas those explants excised from non-pretreated seedlings (i.e., seeds were germinated on hormone-free medium) produced no shoots at all. In another study (Zhang et al. 2001), it was shown 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 1.0, 2.0 or 4.0 mg/l BA. As also suggested in a recent study on quince (*Cydonia oblonga* Mill.), it might be expected that the effects of a growth regulator pretreatment on directing cell competence may differ according to the degree of cell differentiation, thus producing different regenerating responses (D'Onofrio and Morini 2006). This assumption would be in agreement with the competence-determination theory of Christianson and Warnick (1983), who postulated that organogenesis proceeds through three sequential stages; (1) the acquisition of competence (which is believed to be predetermined at an early stage of development) to respond to a particular inductive signal, (2) induction, and (3) morphogenic differentiation and development. In accordance with this theory, we may suggest that the sugar beet tissues obtained from seedlings germinated and then further grown on medium containing BA might have gained a kind of competence at an earlier stage of development, so that their cells can be more readily directed into shoot induction.

When we compared six different *growth* media irrespective of the *germination* and *regeneration* media (Fig. 4), it was clear that those media containing no PGRs were the least effective while those supplemented with a combination of 0.25 mg/l BA and 0.1 mg/l IBA were the most productive; others (0.5, 1.0 or 3.0 mg/l BA and 0.5 mg/l TIBA) being moderately efficient although TIBA seemed to be slightly less effective than BA. On the combined medium (0.25 mg/l BA and 0.10 mg/l IBA), petiole explants of the line EK345 produced the highest mean number of shoots per explant (4.3 shoots) and frequency of organogenic explants (54.2%). Using BA alone at 1.0 or 3.0 mg/l concentrations was similarly effective in both explants types and breeding lines (Fig. 4).

The comparison of five different shoot regeneration media irrespective of the hormonal compositions of germination and growth media revealed that the combination of BA (0.25 mg/l) and IBA (0.10 mg/l) were the most prolific treatment for shoot regeneration from either type of explant of both breeding lines; mean numbers of shoots per explant ranging from 2.0 to 3.4 and mean frequencies of organogenic explants ranging from 20.3 to 55.4% (Fig. 5).

In conclusion, it was evident that both types of explants and breeding lines were able to produce more shoots with higher frequencies of organogenic explants when they were excised from the pretreated seedlings, which had been germinated on medium containing 0.5 mg/l BA for 4 weeks (*first* pretreatment) and further grown on medium containing a combination of 0.25 mg/l BA and 0.10 mg/l IBA for 4 weeks (*second* pretreatment), and cultured on regeneration medium containing again a combination of 0.25 mg/l BA and 0.10 mg/l IBA. Sugar beet is known to be a recalcitrant species with respect to in vitro regeneration, and the enhanced regeneration protocol described in this report is expected to contribute to the efforts of biotechnological improvement of sugar beet, especially through genetic engineering, since the establishment of an efficient and reproducible regeneration protocol is a precondition for a successful genetic transformation study in a given plant species.

Acknowledgments The authors are deeply grateful to the Turkish Sugar Factories Co. for the financial support of this work. Special thanks are also due to Dr. Mustafa Erdal and Dr. Esat Guler (Sugar Institute, Ankara, Turkey) for providing breeding lines.

References

Abogadallah GA, Quick WP (2010) Fast versatile regeneration of *Trifolium alexandrinum* L. Plant Cell Tissue Organ Cult 100:39–48

Arellano J, Fuentes SI, Castillo-Espana P, Hernandez G (2009) Regeneration of different cultivars of common bean (*Phaseolus vulgaris* L.) via indirect organogenesis. Plant Cell Tissue Organ Cult 96:11–18

Christianson ML, Warnick DA (1983) Competence and determination in the process of in vitro shoot organogenesis. Dev Biol 95:288–293

Cogbill S, Faulcon T, Jones G, McDaniel M, Harmon G, Blackmon R, Young M (2010) Adventitious shoot regeneration from cotyledonary explants of rapid-cycling fast plants of *Brassica rapa* L. Plant Cell Tissue Organ Cult 101:127–133

D'Onofrio C, Morini S (2006) Somatic embryo, adventitious root and shoot regeneration in in vitro grown quince leaves as influenced by treatments of different length with growth regulators. Sci Hort-Amsterdam 107:194–199

Detrez C, Tetu T, Sangwan RS, Sangman-Norrel BS (1988) Direct organogenesis from petiole and thin cell layer explants in sugar beet cultured in vitro. J Exp Bot 39:917–926

Detrez C, Sangwan RS, Sangwan-Norrel BS (1989) Phenotypic and karyotypic status of *Beta vulgaris* plant regenerated from direct organogenesis in petiole culture. Theor Appl Genet 77:462–468

Doley WP, Saunders JW (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 Rep 8:222–225

Grieve TM, Gartland KMA, Elliot MC (1997) Micropropagation of commercially important sugar beet cultivars. Plant Growth Regul 21:15–18

Gurel E (1997) Callus and root development from leaf explants of sugar beet (*Beta vulgaris* L.): Variability at variety, plant and organ level. Turk J Bot 21:131–136

Gurel E, Wren MJ (1995a) In vitro development from leaf explants of sugar beet (*Beta vulgaris* L.): Rhizogenesis and the effect of sequential exposure to auxin and cytokinin. Ann Bot-London 75:31–38

Gurel E, Wren MJ (1995b) Measuring polyphenol oxidase activity in small leaf discs of sugar beet (*Beta vulgaris* L.). Turk J Bot 19:497–502

Gurel S, Gurel E, Kaya Z (2001) Callus development and indirect shoot regeneration from seedling explants of sugar beet (*Beta vulgaris* L.) cultured in vitro. Turk J Bot 25:25–33

Gurel E, Topal E, Gurel S (2003a) The effect of pretreating seedlings with BA on direct shoot regeneration from petiole explants of sugar beet (*Beta vulgaris* L.). Biotechnol Biotech Eq 17:89–96

Gurel S, Topal E, Gurel E (2003b) The effect of pretreating seedlings with TDZ on direct shoot regeneration from petiole explants of sugar beet (*Beta vulgaris* L.). Asia-Pac J Mol Biol 11:57–62

Gurel E, Gurel S, Lemaux PG (2008) Biotechnology applications for sugar beet. Crit Rev Plant Sci 27:108–140

Hisano H, Kimoto Y, Hayakawa H, Takeichi J, Domae T, Hashimoto R, Abe J, Asano S, Kanazawa A, Shimamoto Y (2004) High frequency *Agrobacterium*-mediated transformation and plant regeneration via direct shoot formation from leaf explants in *Beta vulgaris* and *Beta maritima*. Plant Cell Rep 22:910–918

Ivic-Haymes SD, Smigocki AC (2005) Identification of highly regenerative plants within sugar beet (*Beta vulgaris* L.) breeding lines for molecular breeding. In Vitro Cell Dev Biol Plant 41:483–488

Jacq B, Tetu T, Sangwan RS, De Laat A, Sangwan-Norreel BS (1993) Efficient production of uniform plants from cotyledon explants of sugarbeet (*Beta vulgaris* L). Plant Breed 110:185–191

Krens FA, Trifonova A, Keizer LCP, Hall RD (1996) The effect of exogenously applied phytohormones on gene transfer efficiency in sugarbeet (*Beta vulgaris* L.). Plant Sci 116:97–106

Kwapata K, Sabzikar R, Sticklen MB, Kelly JD (2010) In vitro regeneration and morphogenesis studies in common bean. Plant Cell Tissue Organ Cult 100:97–105

Li Z, Mize K, Campbell F (2010) Regeneration of daylily (*Hemerocallis*) from young leaf segments. Plant Cell Tissue Organ Cult 102:199–204

Liu CM, Xu ZH, Chua NH (1993) Auxin polar transport is essential for the establishment of bilateral symmetry during early plant embryogenesis. Plant Cell 5:621–630

Liu C, Callow P, Rowland LJ, Hancock JF, Song G (2010) Adventitious shoot regeneration from leaf explants of southern highbush blueberry cultivars. Plant Cell Tissue Organ Cult 103:137–144

Mishutkina YV, Gaponenko AK (2006) Sugar beet (*Beta vulgaris* L.) morphogenesis in vitro: effect of phytohormone type and concentration in the culture medium, type of explants, and plant genotype on shoot regeneration frequency. Russ J Genet 42:150–157

Moghaddam BE, Mesbah M, Yavari N (2000) The effect of in planta TIBA and proline treatment on somatic embryogenesis of sugar beet (*Beta vulgaris* L.). Euphytica 112:151–156

Murashige T, Skoog FA (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497

Ninkovic S, Djordjevic T, Vinterhalter B, Uzelac B, Cingel A, Savic J, Radovic S (2010) Embryogenic responses of *Beta vulgaris* L. callus induced from transgenic hairy roots. Plant Cell Tissue Organ Cult 103:81–91

Ritchie GA, Shoort KC, Davey MR (1989) In vitro shoot regeneration from callus, leaf axils and petioles of sugar beet (*Beta vulgaris* L.). J Exp Bot 40:277–283

Saunders JW, Tsai CJ (1999) Production of somatic embryos and shoots from sugarbeet callus: Effects of abscisic acid, other growth regulators, nitrogen source, sucrose concentration and genotype. In Vitro Cell Dev Biol Plant 35:18–24

Tetu T, Sangwan RS, Sangwan-Norreel BS (1987) Hormonal control of organogenesis and somatic embryogenesis in *Beta vulgaris* callus. J Exp Bot 38:506–517

Thomas TD (2007) Pretreatment in thidiazuron improves the in vitro shoot induction from leaves in *Curculigo orchioides* Gaertn., an endangered medicinal plant. Acta Physiol Plant 29:455–461

Toldi O, Gyulai G, Kiss J, Tarns I, Balazs E (1996) Antiauxin enhanced microshoot initiation and plant regeneration from epicotyl-originated thin layer explants of sugar beet (*Beta vulgaris* L). Plant Cell Rep 15:851–854

Turkish Sugar Co (2010) Sector report 2009, TSFAS, Ankara. http://www.turkseker.gov.tr

Watt MP, Banasiak M, Reddy D, Albertse EH, Snyman SJ (2009) In vitro minimal growth storage of *Saccharum* spp. hybrid (genotype 88H0019) at two stages of direct somatic embryogenic regeneration. Plant Cell Tissue Organ Cult 96:263–271

Zakharchenko NS, Kalyaeva MA, Bur'yanov YI (2000) The method for genetic transformation of different sugar beet varieties. Russ J Plant Physiol 47:70–75

Zhang CL, Chen DF, Elliott MC, Slater A (2001) Thidiazuron-induced organogenesis and somatic embryogenesis in sugar beet (*Beta vulgaris* L). In Vitro Cell Dev Biol Plant 37:305–310

Zhang CL, Chen DF, Kubalakova M, Zhang J, Scott NW, Elliott MC, Slater A (2008) Efficient somatic embryogenesis in sugar beet

(*Beta vulgaris* L.) breeding lines. Plant Cell Tissue Organ Cult 93:209–221

Zhong Z, Smith HG, Thomas TH (1993a) Micropropagation of wild beet (*Beta maritima*) from inflorescence pieces. Plant Growth Regul 12:53–57

Zhong Z, Smith HG, Thomas TH (1993b) In vitro culture of petioles and intact leaves of sugar beet (*Beta vulgaris*). Plant Growth Regul 12:59–66