In vitro Explant Growth and Shoot Regeneration from Petioles of Sugar Beet (*Beta vulgaris* L.) Lines at Different Ploidy Levels

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ABSTRACT

This study was carried out to compare 'ELK 345' (diploid) and 'CBM 315' (tetraploid) sugar beet lines with respect to *in vitro* explant growth, shoot regeneration capacity of petiole explants, *in vitro* rooting and plantlet establishment. Petioles were cultured on MS (Murashige and Skoog) medium supplemented with 1 mg l^{-1} 6-benzylaminopurine (BAP) and 0.2 mg l^{-1} naphthaleneacetic acid (NAA). Regenerated shoots were successfully rooted on MS medium containing 3 mg l^{-1} indole-3-butyric acid (IBA). It was observed that petiole explants of the tetraploid line 'CBM 315' gave rise to the highest scores with respect to explant fresh and dry weights, shoot regeneration percentage, shoot number per petiole, shoot length, total shoot number per Petri dish, successful rooting and plantlet establishment. Petiole explants of diploid line ('ELK 345') produced 12.61 shoots per explants while petioles of tetraploid line ('CBM') produced 20.23 shoots per explant.

Additional key words: *In vitro* explant growth, shoot regeneration, *Beta vulgaris*, ploidy level

Sugar beet (*Beta vulgaris* L.), important sucrose-producing crop worldwide in temperate regions, supplies about 20% of the sugar consumption (Turkish Sugar Co., 2010). Developing new sugar beet varieties with conventional plant breeding methods is slow and labor intensive. Tissue culture methods integrated with conventional breeding programs are playing an increasingly significant role in the improvement of sugar beet (D'Halluin et al., 1992; Gurel, 2000; Hisano et al., 2004). Advanced *in vitro* culture and genetic transformation technologies have been incorporated with classical breeding programs of sugar beet, aiming at the production of herbicide- and salt-tolerant, disease- and pest-resistant cultivars (Tenning, 1998; Shimamoto and Domae, 1999; Gurel et al., 2001; Yang et al., 2005; Gurel et al., 2008). However, sugarbeet is recalcitrant with respect to *in vitro* culture and genetic transformation (Tetu et al., 1987; Krens et al., 1989; Gurel et al., 2008).

Genetic variation is a prerequisite for successful plant breeding. *In vitro* culture techniques seem to offer certain advantages in this respect through somatic hybridization, induction of mutants and selection of disease free and disease resistant plants (Thirugnanakumar et al., 2009). Any progress in increasing the adventitious shoot regeneration frequency in sugar beet *in vitro* culture is extremely valuable for the development of new cultivars. To our knowledge, there is no report on the effect of ploidy level on *in vitro* tissue culture response in sugar beet. Thus, this study was conducted to examine the effect of the ploidy level on *in vitro* explant's growth, adventitious shoot regeneration, rooting and plantlet establishment from petiole explants of two sugar beet (*Beta vulgaris* L.) lines.

MATERIALS AND METHODS

Plant material

Two sugar beet (*Beta vulgaris* L.) breeding lines, 'ELK 345' (diploid multigerm, good root yield, good sugar yield) and 'CBM 315' (tetraploid multigerm, good root yield, good sugar yield) were used as sources of explants. All breeding lines which were obtained from the Sugar Research Institute, Etimesgut, Ankara, were originated from inbred lines.

Surface sterilization and germination of seeds

Seeds were placed in sterile bottles having 100% commercial bleach (5% sodium hypochlorite) and were shaken for 5 h at room temperature. This was followed by 3-4 washes with sterile water and a 24 h rinse in sterile water to increase permeability of the true seed coat as reported by Yildiz et al. (2007). After sterilization, the seed coat was removed from the sterile true seeds. Removal of the seed coat enabled the true seeds to germinate more rapidly. Sterilized seeds were germinated in babyfood-jars containing 30 ml of MS medium (Murashige and Skoog, 1962) supplemented with 3% sucrose (w/v), 0.7% (w/v) agar,

0.5 mg l^{-1} 2,3,5-triiodobenzoic acid (TIBA), and 1 mg l^{-1} BAP for promoting organogenic cell lines in cultured explants (Jack et al., 1992).

Explant source and culture conditions

Petiole explants (0.7 mm in length) were excised from 8-week-old sterile seedlings. For shoot regeneration, petioles were cultured for 5 weeks on MS medium supplemented with 1 mg l^{-1} BAP and 0.2 mg l^{-1} NAA at 1% sucrose as reported by Yildiz et al. (2007). Petioles were submerged in sterile distilled water with a gentle shaking for 20 min before placing on regeneration medium to increase the adventitious shoot regeneration frequency as reported by Yildiz and Özgen (2004). The pH of the medium was adjusted to 5.8 and autoclaved at 120ºC for 20 min. Growth regulators were added to the media before autoclaving.

All cultures were incubated under a cool white fluorescent light (27 µmol m^{-2} s^{-1}) with a 16 h light/8 h dark photoperiod in a growth chamber at 25±1°C.

Observations

Cell size, measured by width and cell length, was recorded in µm using a microscope at 60X magnification in the petiole's epidermis layer of sterile seedlings of diploid and tetraploid lines before culture initiation. Fresh and dry weights of petioles with shoots were calculated at the end of the culture. Petiole segments were weighed to determine the fresh weight. The dry weight was obtained after drying explants at 105ºC for 2 h. All measurements were made using an analytical scale, with precision of 0.001 g. Data collected included shoot regeneration percentage, shoot number per petiole, shoot length, total shoot number per Petri dish, number of shoots rooted and percentage of shoots rooted.

Rooting and transplanting

Seventy regenerated shoots from diploid and tetraploid lines were transferred to sterile baby food-jars containing 30 ml MS medium with 3 mg l^{-1} IBA; they were incubated for two weeks at 25±1°C to induce root formation. Rooted shoots were then transferred to pots in a growth room for two weeks where light, temperature and humidity were controlled. Humidity was decreased gradually from 100% to 40% during two weeks for acclimatization of seedlings. After two weeks, plantlets were moved to a greenhouse for 10 days and finally they were transplanted to the field.

Statistical analysis

In the study, three replicates were used. Petri dishes (100 x 10 mm) containing 10 explants were considered the experimental units. The study was set in three parallels to confirm the accuracy of the study. Data were statistically analyzed by Independent-Samples t test in the 'SPSS for Windows' program. Values presented in percentages were

transformed using an arcsine transformation before statistical analysis (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

Tissue necrosis, which is caused by the oxidation of phenolic compounds by several enzymes such as polyphenoloxidase (PPO) and peroxidase (POD), is one of the main problems in sugar beet tissue culture (Whitaker and Lee, 1995; Yildiz et al., 1997; Gurel et al., 2001). Because phenolic compounds have been found in different beet tissues (Wende et al., 1999; Kaur and Kapoor, 2002), sugar beet has been placed in a high phenolic concentration group. However, the protocol described by Yildiz et al. (2007) has eliminated the problem of tissue necrosis in sugar beet *in vitro* culture. Consequently high frequency shoot regeneration, which is a prerequisite for an efficient transformation system, was achieved. In the current study this protocol was used successfully to obtain adventitious shoots.

We used petioles as an explant and 1 mg l^{-1} BAP and 0.2 mg l^{-1} NAA as the combination of growth regulators for shoot regeneration because studies reported the petiole was the most responsive explant for *in vitro* culture of sugar beet and the most effective combination of the plant growth regulators was 1 mg l^{-1} BAP and 0.2 mg l^{-1} NAA (Tetu et al., 1987; Detrez et al., 1988, 1989; Freytag et al., 1988; Ritchie et al., 1989; Toldi et al., 1996; Grieve et al., 1997; Zhang et al., 2001; Yildiz et al., 2007).

There are differences between diploid and polyploid plants from morphological, physiological, cellular and biochemical aspects (Berkov and Philipov, 2002). Berkov and Philipov (2002) have reported that the roots and leaves of tetraploid *Datura stramonium* plants had a higher alkaloid content than diploid plants. It was reported that the number of chlorophyll-containing chloroplasts increased from diploids to tetraploids in black wattle (Beck et al., 2003). The overall chlorophyll content in the diploids of the black wattle was 40% less than that of the tetraploids (Mathura et al., 2006). In alfalfa, chlorophyll content and other proteins were shown to almost double from diploid to tetraploid plants (Molin et al., 1982). Similarly, it was reported that in C_4 grass *Panicum virgatum*, chlorophyll content and other soluble proteins were found to be 40-50% higher in octaploids than in tetraploids (Warner et al., 1987). Yildiz et al. (2005) conducted a study of two sugar beet lines (CBM 315-tetraploid and ELK 345-diploid) at different ploidy levels to determine *in vitro* susceptibility to *Agrobacterium tumefaciens* infection. This study showed statistically significant differences in all parameters examined between lines of different ploidy levels (Tables 1 through 4).

In our study, data related to the cell size were obtained in µm using a microscope at 60X magnification on the petiole's epidermis layer of the sterile seedlings of diploid and tetraploid lines before culture ini-

Table 1. Cell sizes in the epidermis layer of petioles of sterile seedlings of diploid and tetraploid lines.

	Cell size			
	Cell width (µm)		Cell length (µm)	
	'ELK 345' Diploid	'CBM 315' Tetraploid	'ELK 345' Diploid	'CBM 315' Tetraploid
1st experiment	33.84±1.657	49.04±2.162	95.52±2.239	138.24±2.274
t value	5.580**		13.387**	
2nd experiment	36.72±1.143	50.46±1.739	88.62±1.709	132.62±2.045
t value	6.603**		16.512**	
3rd experiment	31.08±2.425	46.42±1.550	98.32±2.281	145.12±2.787
t value	5.329**		12.996**	
Mean[1]	33.88	48.64	94.15	138.66

Significantly different from zero at ** $p < 0.01$
[1] Mean of three experiments

tiation. The largest cells measured were from the tetraploid line in all cases. The differences between cell sizes of the diploid and tetraploid lines were statistically significant at the 0.01 level. The largest mean cell width recorded was 48.64 µm in the tetraploid line, and was 33.88 µm in the diploid line (Table 1). Similarly, the largest mean cell length obtained was 138.66 µm in the tetraploid line, and 94.15 µm in the diploid line. From the results, it was evident that both parameters relating cell size were almost 50% larger in the tetraploid line in all cases (Table 1). Our finding were verified by Smith et al. (2003) reporting that polyploidy increases the cell size and volume of perennial ryegrass (*Lolium perenne* L.).

The tetraploid line 'CBM 315' had a significantly higher fresh weight ($p < 0.05$) than the diploid line 'ELK 345' in all three experiments (Table 2). Dry weights also were higher in the tetraploid line, and the differences between these lines were statistically significant at 0.01 level in all experiments (Table 2). During culture, petiole explants of the tetraploid line were observed to grow faster than the ones

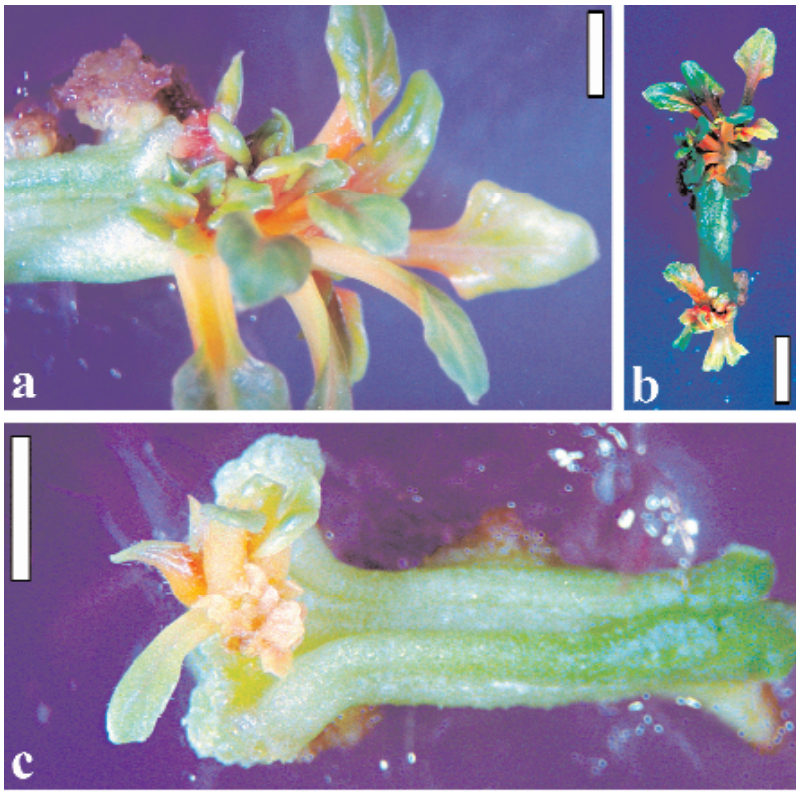
Table 2. Fresh and dry weights, water and dry matter contents of petiole explants of 'ELK 345' (diploid) and 'CBM 315' (tetraploid) lines 5 weeks after culture initiation on MS medium containing 1 mg l^{-1} BAP and 0.2 mg l^{-1} NAA

	Fresh weight (g)		Dry weight (g)		Water content (%)		Dry matter content (%)	
	'ELK 345' Diploid	'CBM 315' Tetraploid	'ELK 345' Diploid	'CBM 315' Tetraploid	'ELK 345' Diploid	'CBM 315' Tetraploid	'ELK 345' Diploid	'CBM 315' Tetraploid
1st experiment	0.168±0.009	0.226±0.011	0.012±0.001	0.023±0.002	92.83±0.410	89.76±0.410	7.17±1.147	10.24±1.147
t value	4.226*		4.621**		2.561*		2.561*	
2nd experiment	0.161±0.091	0.251±0.028	0.012±0.001	0.022±0.002	92.34	91.34	7.66	8.66
t value	3.120*		4.542**		2.165ns		2.165ns	
3rd experiment	0.187±0.017	0.284±0.019	0.013±0.0003	0.023±0.001	92.76	91.77	7.24	8.23
t value	3.794*		6.708**		1.401ns		1.401ns	
Mean[1]	0.172	0.254	0.012	0.023	92.64	90.96	7.36	9.04

Significantly different from zero at * $p < 0.05$ and ** $p < 0.01$

[1] Mean of three experiments

Fig. 1. *In vitro* shoot regeneration from petiole explants of **(a-b)** 'CBM 315' (tetraploid) and **(c)** 'ELK 345' (diploid) line 5 weeks after culture initiation. (Bars= 0.5 cm in a and c, 1.0 cm in b)



of the diploid line. By the end of the culture, petiole explants of the tetraploid line were larger, well developed, and the number of shoots regenerated also was higher (Figure 1a-b) than the diploid line (Figure 1c). The largest mean fresh and dry weights of petiole explants were from the tetraploid line – 0.254 g and 0.023 g, respectively (Table 2). In the diploid line, the mean fresh and dry weights of petioles were 0.172 g and 0.012 g, respectively (Table 2). The difference between fresh and dry weights signifies the tissue water content. From these results, the tissue water content was calculated as 0.231 g (0.254-0.023) in the tetraploid line 'CBM 315', and 0.160 g (0.172-0.012) in the diploid line 'ELK 345'.

The cells with higher ploidy levels in a yeast, *Candida* sp., have larger vacuoles (Jibiki et. al., 1993); vacuoles play an important role in regulating the osmotic pressure of the cell (Guertin and Sabatini, 2005). Higher osmotic pressure in polyploid tomato plant cells, as re-

ported by Tal and Gardi (1976), could cause higher tissue metabolic activity by increasing water and hormone uptake from the medium. Cell enlargement by water absorption, cell vacuolation, and turgor-deriven wall expansion is the main reason of fresh weight increase, as reported by Dale (1988). Increase in dry weight has been related closely to cell division and new material synthesis (Sunderland, 1960). Thus, increase in the fresh and dry weights of petiole explants of the tetraploid line at the end of culture in our study most likely were due chiefly to an increase in the absorption of water and other components from the basal medium via the higher cellular osmotic pressure. Additionally, lower osmotic pressure of the cells of diploid line probably corrolated to lower fresh and dry weights of petioles by decreasing the absorption of water and other components from the medium. Tissue water content results indicated that the tetraploid line had higher osmotic pressure, which most likely caused higher absorption of water and other components from the growth. Higher results of all parameters in our study could be attributed to higher cell osmotic pressure of the tetraploid line 'CBM 315'. Yildiz and Özgen (2004) have reported that increasing tissue water content, which caused higher tissue metabolic activity, resulted in higher results of all parameters examined in flax (*Linum usitatissimum*).

It has been shown that increase in ploidy level leads to a larger cell that has a higher growth rate (Jibiki et.al., 1993). Tetraploid genotypes had a higher water content (Tal and Gardi, 1976) and more organic solutes than diploid genotypes (Reinink and Biom-Zandstra, 1989). Warner and Edwards (1989) have reported that the chromosome number determines the size of leaves, the size of cells, the number of chloroplasts per cell, and amounts of photosynthetic enzymes and pigments in cell. As the chromosome number increased, DNA content per cell, enzyme activity per cell, cell volume, and photosynthesis per cell all increased. It also was reported that the photosynthetic capacity of larger cells in polyploid plants are higher than smaller cells with lower chromosome numbers (Molin et al., 1982; Warner et al., 1987; Wintermans and De Mots, 1965).

In all of our experiments, the highest results were obtained in the parameters of shoot regeneration percentage, shoot number per petiole, shoot length, total shoot number per Petri dish, number of shoots rooted, and the percentage of shoots rooted from petiole explants of the tetraploid line. The differences between petiole explants of the diploid and tetraploid lines for all parameters examined were statistically significant at $p < 0.01$, with the exception of shoot regeneration percentage, which were significantly different in all experiments at $p < 0.05$ (Table 3, Table 4, Figs. 1, 2).

Shoot primordia on petiole explants appeared in the first week of the culture in the tetraploid line but they developed 16 days after culture initiation in the diploid line. The highest mean shoot regeneration percentage and mean shoot number per petiole was recorded as 69.99% and 20.23 in the tetraploid line and they were 45.57% and

Table 3. Adventitious shoot regeneration from petiole explants of 'ELK 345' (diploid) and 'CBM 315' (tetraploid) lines 5 weeks after culture initiation on MS medium containing 1 mg l^{-1} BAP and 0.2 mg l^{-1} NAA

	Shoot regeneration (%)		Shoot number per petiole		Shoot length (cm)		Total shoot number per Petri dish	
	'ELK 345' Diploid	'CBM 315' Tetraploid	'ELK 345' Diploid	'CBM 315' Tetraploid	'ELK 345' Diploid	'CBM 315' Tetraploid	'ELK 345' Diploid	'CBM 315' Tetraploid
1st experiment	40.0±5.773	66.7±3.333	12.53±0.617	22.80±1.106	2.2±0.577	3.2±0.153	50.12±9.1811	152.73±14.589
t value	3.957*		8.105**		6.124*		5.917**	
2nd experiment	46.7±3.333	70.0±5.774	13.70±0.656	19.70±0.929	1.9±0.153	2.7±0.058	63.67±3.844	138.97±12.305
t value	3.368*		5.276**		3.703**		4.104**	
3rd experiment	50.0±5.774	73.3±3.333	11.60±0.625	18.20±0.557	1.8±0.100	2.5±0.115	58.70±9.650	133.60±8.426
t value	3.501*		7.889**		4.583**		5,847**	
Mean[1]	45.57	69.99	12.61	20.23	2.0	2.8	57.50	141.77

Significantly different from zero at * $p < 0.05$ and ** $p < 0.01$

[1] Mean of three experiments

Fig. 2. Two-week-old rooted seedlings derived from **(a)** 'CBM 315' (tetraploid) and **(b)** 'ELK 345' (diploid) line. (Bar = 1.5 cm)

12.61 in the diploid line (Table 3). Regenerated shoot length was found to be higher in the tetraploid line 5 weeks after culture initiation. The mean shoot length was 2.8 cm in the tetraploid line, and it was 2.0 cm in the diploid line (Table 3).

The mean total shoot number per Petri dish, which can be determined by shoot regeneration percentage and shoot number per petiole, was recorded as 141.8 in the tetraploid line and 57.5 in the diploid line (Table 3). Shoots regenerated from petiole explants of the diploid and tetraploid lines were rooted on MS medium containing 3 mg l^{-1} IBA for 2 weeks. The best results were obtained in shoots regenerated from petiole explants of the tetraploid line in all three experiments (Table 4). From the results, it was evident that plantlets grown from petioles of tetraploid line were more vigorous and larger than the plantlets grown from petioles of the diploid line (Fig. 2a). Of the 70 shoots transferred to rooting medium, 61.3 shoots (87.6%) from tetraploid line 'CBM 315' and 51.7 shoots (73.8%) from the diploid line 'ELK 345' were rooted successfully (Table 4). Transferred plants reached harvest ma-

Table 4. *In vitro* root development of shoots regenerated from petiole explants of 'ELK 345' (diploid) and 'CBM 315' (tetraploid) lines on rooting medium enriched with 3 mg l^{-1} IBA 2 weeks after culture initiation.

	Number of shoots rooted		**% of shoots rooted**	
	'ELK 345' Diploid	**'CBM 315' Tetraploid**	**'ELK 345' Diploid**	**'CBM 315' Tetraploid**
1st experiment	53±1.155	64±1.000	75.71±1.648	91.43±1.430
t value	7.201**		6.662**	
2nd experiment	52±1.528	59±1.155	74.28±2.181	84.29±1.648
t value	3.656*		3.709*	
3rd experiment	50±1.528	61±1.399	71.43±2.181	87.14±1.648
t value	5.745**		5.692**	
Mean[1]	51.7	61.3	73.81	87.62

Significantly different from zero at * $p < 0.05$ and ** $p < 0.01$
[1] Mean of three experiments

turity in the field and no morphological abnormalities were observed.

To our knowledge, this is the first report of the effect of ploidy levels on tissue culture response, rooting, and plantlet establishment *in vitro*. Superiority of the performance of the tetraploid sugar beet line ('CBM 315') in our study with respect to tissue culture response agreed with the results of Yildiz et al. (2007). In the study conducted by Yildiz et al. (2007) in sugar beet, two diploid lines ('ELK 345' and 'M 114') and one tetraploid line ('CBM 315') were used to evaluate the effect of different sucrose concentrations in the medium on tissue necrosis and shoot regeneration capacity of the explant. They reported that tetraploid line gave rise to the more shoots per explant than did the two diploid lines. Our findings were in agreement with Stebbins (1947) who reported that tetraploids have higher vegetative growth.

LITERATURE CITED

Beck, S.L., R.W. Dunlop, and A. Fossey. 2003. Stomatal length and frequency as a measure of ploidy level in black wattle, *Acacia mearnsii* (De Wild.). Bot. J. Linn. Soc. 141: 177-181.

Berkov, S. and S. Philipov. 2002. Alkoloid production in diploid and autotetraploid plants of *Datura stramonium*. Pharm. Biol. 40: 617-621.

Dale, J.E. 1988. The control of leaf expansion. Annu. Rev. Plant Physiol. 39: 267-295.

D'Halluin, K., M. Bossut, E. Bonner, B. Mazur, J. Leemans and J. Botterman. 1992. Transformation of sugar beet (*Beta vulgaris* L.) and evaluation of herbicide resistance in transgenic plants. Biotechnology 10: 309-314.

Detrez, C., T. Tetu, R.S. Sangwan and B.S. Sangwan-Norreel. 1988. Direct organogenesis from petiole and thin cell layer explants in sugar beet cultured *in vitro*. J. Exp. Bot. 39: 917-926.

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

Freytag, A.H., S.C. Anand, A.P. Rao-Arelli and L.D. Owens 1988. An improved medium for adventitious shoot formation and callus induction in *Beta vulgaris* L. *in vitro*. Plant Cell Rep. 7: 30–34.

Grieve, T.M., K.M.A. Gartlan and M.C. Elliott. 1997. Micropropagation of commercially important sugar beet cultivars. Plant Growth Regul. 21: 15-18.

Guertin, D.A. and D.M. Sabatini. 2005. Cell size control. Encyclopedia of Life Sciences. John Wiley & Sons Ltd.

Gurel, S. 2000. *In vitro* growth and development of sugar beet (*Beta vulgaris* L.) from callus, ovule, suspension cells and protoplasts. PhD Dissertation, Middle East Technical University, Ankara, Turkey.

Gurel, S., E. Gurel and Z. Kaya. 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., S. Gurel and P.G. Lamaux. 2008. Biotechnology applications for sugar beet. Crit. Rev. Plant Sci. 27: 108-140.

Hisano, H., Y. Kimoto, H. Hayakawa, J. Takeichi, T. Domae, R. Hashimoto, J. Abe, S. Asano, A. Kanazawa and Y. Shimamoto. 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.

Jack, B., T. Tetu, R.S. Sangwan, A.D. Laat and B.S. Sangwan-Norreel. 1992. Plant regeneration from sugarbeet (*Beta vulgaris* L.) hypocotyls cultured *in vitro* and flow cytometric nuclear DNA analysis of regenerants. Plant Cell Rep. 11: 329-333.

Jibiki, M., Y. Kuno, H. Shinoyama and T. Fujii. 1993. Isolation and properties of large cell strains from a methanol-utilizing yeast, *Candida* sp. N-16 by colchicine treatment. J. Gen. Appl. Microbiol. 39: 439-442.

Kaur, C. and H.C. Kapoor. 2002. Anti-oxidant activity and total phenolic content of some Asian vegetables. Int. J. Food Sci. Tech. 37: 153-161.

Krens, F.A. and D. Jamar. 1989. The role of explant source and culture conditions on callus induction and shoot regeneration in sugarbeet (*Beta vulgaris* L.). J. Plant Physiol. 134: 651-655.

Mathura, S., A. Fossey and S. Beck. 2006. Comparative study of chlorophyll content in diploid and tetraploid black wattle (*Acacia mearnsii*). Forestry 79: 381-388.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 15: 473-479.

Molin, WT., S.P. Meyers, G.R. Baer and L.E. Schrader. 1982. Ploidy effects of isogenic populations of alfalfa II. Photosynthesis, chloroplast number, ribulose-1,5-bisphosphate carboxylase, chlorophyll, and DNA in protoplasts. Plant Physiol. 70: 1710-1714.

Reinink, K. and M. Biom-Zandstra. 1989. The relation between cell size, ploidy level and nitrate concentration in lettuce. Physiol. Plant. 76: 575-580.

Ritchie, G.A., K.C. Short and M.R. Davey. 1989. *In vitro* shoot regeneration from callus, leaf axils and petioles of sugarbeet (*Beta vulgaris* L.). J. Exp. Bot. 40: 277-283.

Shimamoto, Y. and T. Domae 1999. Resistance to larvae of cabbage armyworm (*Mamestra brassicae* L.) in ICP gene transductant of sugarbeet. Proc. Jpn. Soc. Sugar Beet Technol. 41: 90–98.

Smith, K.F., N.M. McFarlane, V.M. Croft, P.J. Trigg and G.A. Kearney. 2003. The effects of ploidy and seed mass on the emergence and early vigour of perennial ryegrass (*Lolium perenne* L.) cultivars. Aust. J. Exp. Agr. 43: 481-486.

Snedecor, G.W. and W.G. Cochran. 1967 Statistical Methods. The Iowa State University Press, Iowa, USA.

Stebbins, G.L. 1947. Types of polyploids: their classification and significance. Adv. Genet. 1: 403–429.

Sunderland, N. 1960. Cell division and expansion in the growth of the leaf. J. Exp. Bot. 11: 68-80.

Tal, M. and I. Gardi. 1976. Physiology of polyploid plants: Water balance in autotetraploid and diploid tomato under low and high salinity. Physiol. Plant. 38: 257-261.

Tenning, P. 1998. Transgenic herbicide tolerant sugar beet — Present status and future developments. Aspects Appl. Biol. 52: 273-278.

Tetu, T., R.S. Sangwan and B.S. Sangwan-Norreel. 1987. Hormonal control of organogenesis and somatic embryogenesis in *Beta vulgaris* callus. J. Exp. Bot. 38: 506-517.

Thirugnanakumar, S., K. Manivannan, M. Prakash, R. Narasimman and Y. Anitha Vasline. 2009. In Vitro Plant Breeding. Agrobios, India, 287 p.

Toldi, O., G. Gyulai, J. Kiss, I. Tarns and E. Balazs. 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, Turkey. http://www.turkseker.gov.tr

Warner, D.A., M.S.B. Ku and G.E. Edwards. 1987. Photosynthesis, leaf anatomy, and cellular constituents in the polyploidy C_4 grass *Panicum virgatum*. Plant Physiol. 84: 461-466.

Warner, D.A. and G.E. Edwards. 1989. Effects of polyploidy on photosynthetic rates, photosynthetic enzymes, contents of DNA, chlorophyll, and sizes and numbers of photosynthetic cells in the C_4 dicot *Atriplex confertifolia*. Plant Physiol. 91: 1143-1151.

Wende, G., K.W. Waldron, A.C. Smith and C.T. Brett. 1999. Developmental changes in cell-wall ferulate and dehydrodiferulates in sugar beet. Phytochemistry 52: 819-827.

Wintermans, J.F.G.M. and A. De Mots. 1965. Spectrophotometric characteristics of chlorophylls *a* and *b* and their pheophytins in ethanol. Biochim. Biophys. Acta. 109: 448-453.

Whitaker, J.R. and C.Y. Lee. 1995. Recent advances in chemistry of enzymatic browning. In: Lee, C.Y., Whitaker, J.R. (Eds.), Enzymatic Browning and Its Prevention, Washington, DC, USA, ACS Symp. Series 600, pp 2-7.

Yang, A.F., X.G. Duan, X.F. Gu, F. Gao and J.R. Zhang 2005. Efficient transformation of beet (*Beta vulgaris*) and production of plants with improved salt-tolerance. Plant Cell Tiss. Org. Cult. 83: 259–270.

Yildiz, M., M. Avci and M. Ozgen. 1997. Studies on sterilization and medium preparation techniques in sugarbeet (*Beta vulgaris* L.) regeneration. Deutsch-Türkische Agrarforschung Symposium, Antalya, Turkey, pp 125-130.

Yildiz, M. and M. Ozgen. 2004. The effect of a submersion pretreatment on in vitro explant growth and shoot regeneration from hypocotyls of flax (*Linum usitatissimum*). Plant Cell Tiss. Org. 77: 111-115.

Yildiz, M., N. Koyuncu and M. Ozgen. 2005. *In vitro* susceptibility of two sugar beet (*Beta vulgaris* L.) lines in different ploidy levels to *Agrobacterium tumefaciens*. The Sixth Field Crops Congress of Turkey, Antalya, Turkey, pp 1089-1093.

Yildiz, M., S. Onde and M. Ozgen. 2007. Sucrose effects on phenolic concentration and plant regeneration from sugarbeet leaf and petiole explants. J. Sugar Beet Res. 44: 1–15.

Zhang, C.L., D.F. Chen, M.C. Elliott and A. Slater. 2001. Thidiazuroninduced organogenesis and somatic embryogenesis in sugar beet (*Beta vulgaris* L). In Vitro Cell. Dev. Biol.-Plant 37: 305–310.