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Research Article

Establishment of Cell Suspension Cultures and Plant Regeneration in Sugar Beet (*Beta vulgaris* L.)

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Abstract: The establishment of cell suspension culture from the callus of sugar beet (*Beta vulgaris* L.) breeding lines and plant regeneration from suspension-derived callus are described in this study. Using different concentrations and combinations of BAP and 2,4-D, the growth patterns of cell suspension cultures were examined during a range of culture periods (0, 3, 5, 7, 9, 11, 13 and 15 days). In all lines, the growth rates of cells were initially slow but as the culture proceeded, they increased significantly and accumulated great amounts of biomass over a period of 15 days. Medium containing high BAP (0.25 mg/l) and 2,4-D (0.25 mg/l) induced higher rates of cell division than the medium containing low BAP (0.1 mg/l) and 2,4-D (0.1 mg/l) or the control. A genotypic variation was evident when the mean growth rates of different lines were compared, with lines M114, ELK345, ÇBM315 and M1017 producing a mean rate of 34.8%, 21.5%, 19.2% and 13.4%, respectively. In all lines, approximately 50% of the suspension-derived callus formed shoots when cultured on medium containing BAP and IAA, or TDZ alone. Rooting was readily achieved when shoots were incubated on medium containing 3.0 mg/l IBA.

Key Words: Sugar beet, suspension culture, plant regeneration, breeding lines

Şeker Pancarında (*Beta vulgaris* L.) Hücre Süspansiyon Kültürlerinin Oluşturulması ve Bitki Rejenerasyonu

Özet: Bu çalışmada, şeker pancarı (*Beta vulgaris* L.) ıslah hatlarından elde edilen kallustan süspansiyon kültürlerinin oluşturulması ve süspansiyon-kökenli kallustan bitki rejenerasyonu tanımlanmıştır. BAP ve 2,4-D'nin farklı konsantrasyon ve kombinasyonlarını kullanarak, kültürlerin büyüme motifleri değişik inkübasyon süreleri boyunca (0, 3, 5, 7, 9, 11, 13 ve 15 gün) incelenmiştir. Süspansiyon kültüründeki hücrelerin büyüme oranları, kültür başlangıcında bütün hatlarda yavaş olmuş fakat takip eden günlerde önemli artışlar göstererek 15. günün sonunda büyük miktarlarda biyomas birikimi sağlanmıştır. Yüksek BAP (0.25 mg/l) ve 2,4-D (0.25 mg/l) içeren ortamlar, düşük BAP (0.1 mg/l) ve 2,4-D (0.1 mg/l) içeren ortamlara veya kontrol ortamına göre daha yüksek oranlarda hücre bölünmesine yol açmıştır. Hatların ortalama hücre büyüme oranları karşılaştırıldığında, genotipik bir varyasyonun var olduğu gözlenmiştir; ıslah hattı M114, ELK345, ÇBM315 ve M1017'ye ait ortalama hücre büyüme oranları, sırasıyla, %34.8, %21.5, %19.2 ve %13.4 olmuştur. Bütün hatlarda, elde edilen süspansiyon-kökenli kallusların yaklaşık %50'si, BAP+IAA veya sadece TDZ içeren ortamda inkübe edildiğinde sürgün oluşturmuştur. Sürgünlerin köklendirilmesi ise, sadece 3.0 mg/l IBA içeren ortamda kolaylıkla gerçekleşmiştir.

Anahtar Sözcükler: Şeker pancarı, süspansiyon kültürü, bitki rejenerasyonu, ıslah hatları

Introduction

Callus tissue is an essential material in plant cell culture systems. When it is introduced into a liquid medium and agitated, the cells disperse throughout the liquid to form a cell suspension culture. Such cells are, in theory, totipotent and should also have a potential to synthesize any of the compounds normally associated with the intact plant (Allan, 1996). As new cells are formed they are dispersed into the liquid medium and become clusters and aggregates. Cells in suspension can exhibit much higher rates of cell division than do cells in callus culture. Thus, cell suspension offers advantages

when rapid cell division or many cell generations are desired, or when a more uniform treatment application is required (Philips et al., 1995).

The techniques of plant cell culture facilitate the rapid production of variant cell lines via selection procedures, very similar to those employed in microbial systems. These variant cell lines are useful for research into the genetics and biochemistry of plant cells and also in biotechnology for the production of new plant varieties and secondary metabolites. Rapidly growing, fine suspension cultures or friable calluses are generally the most suitable for selection purposes. Where it is possible to regenerate plants from variant cells, selection techniques have potential for the production of crop varieties with new characteristics such as herbicide resistance (Saunders et al., 1992), salt tolerance (Freytag et al., 1990), cold tolerance, disease resistance and metal tolerance (Cresswell, 1995). The diverse group of compounds known as plant secondary metabolites include subtances with pharmaceutical activity (e.g., morphine, vinvristine), fragrances, pigments, latex, enzymes and carbohydrates (Hunter & Kilby, 1990). Sugar beet cells are used for obtaining the pigment betalains (Weller & Lasure, 1981), betanin (Hunter & Kilby, 1990), betaxanthin and betacyanin (Hamill et al., 1986; Giord & Zryd, 1991). Cell suspensions have also proven to be excellent starting materials for the isolation of protoplasts to be used in a wide range of applications including cell fusion and genetic manipulation (Hall, 1991).

In this study, we describe the establishment of cell suspension cultures from white and friable callus, which was previously obtained from several sugar beet breeding lines and designated as "Type I" callus (Gürel et al., 2001). Using different concentrations and combinations of the cytokinin BAP and auxin 2,4-D, the growth patterns of the cultures were examined during a range of culture durations. The optimized culture conditions obtained from the present study will then be used in our protoplast culture and somatic hybridization experiments.

Materials and Methods

Plant Material and Culture Conditions

The establishment of sugar beet cell suspension cultures is summarized, stage by stage, in Table 1. Type I callus obtained from callus cultures of breeding lines M114, ELK345, ÇBM315 and M1017 (Gürel et al., 2001) was used for culture initiation. Approximately 1 g of callus, which was still in its active growth phase (i.e., after the 15th day of subculture), was placed in 250 ml flasks containing 50 ml liquid MS (Murashige & Skoog, 1962) medium supplemented with either no plant growth regulators or with 0.1 or 0.25 mg/l BAP in combinations with 0.1 or 0.25 mg/l 2,4-D (see Tables 3-6). Cultures were incubated in complete darkness at 25°C on a horizontal shaker at 100 rpm for 15 days. Growth of cells was then determined by measuring packed cell volume (PCV) described by Allan (1996) under sterile conditions at 0, 3, 5, 7, 9, 11, 13 and 15 days of culture.

Table 1. An experimental outline for *(A)* the initiation of suspension cultures from white and friable callus, *(B)* cell growth measured by PCV, *(C)* callus induction from suspension cells, *(D)* subculture of callus initiated from suspension cells and finally, *(E)* shoot regeneration from the subcultured callus obtained from the suspension cells.

Stages	Process	Culture Conditions
A. Initiation	White and friable callus obtained from lines M114, ELK345, ÇBM315 and M1017	(see Gürel et al., 2001)
B. Cell Growth	1 g callus into liquid MS medium containing 0.1 or 0.25 mg/l BAP and 0.1 or 0.25 mg/l 2,4-D	15 days in darkness at 25°C
C. Callus Induction	Solid MS medium containing 0.3 mg/l BAP and 0.1 mg/l IAA	15 days in darkness at 25°C
D. Callus Subculture	Solid MS medium containing either i) 0.3 mg/l BAP and 0.1 mg/l IAA, or ii) 2.5 mg/l TDZ only	15 days in darkness at 4°C
E. Shoot Regeneration	Solid MS medium containing either i) 1.0 mg/l BAP and 0.3 mg/l IAA, or ii) 2.5 mg/l TDZ only	30 days at 25°C in 16 h light/8 h darkness

For PCV measurements, which were repeated three times per treatment, the suspension cells in flasks were gently shaken and then 10 ml aliquots were transferred into graduated conical centrifuge tubes followed by centrifugation at 200 g for 5 min using a swing-out rotor (Allan, 1996).

Suspension cultures were subcultured weekly by replacing 30 ml of the total 50 ml culture in the flask with fresh culture medium. For callus formation from the suspension cultures, 15-day-old suspension cells were used as follows. After decanting the liquid medium, cells in flasks were collected and washed three times in sterile distilled water and transferred to solid MS medium containing 0.3 mg/l BAP and 0.1 mg/l IAA followed by incubation in darkness at 25°C for two weeks. Then the callus was transferred to solid MS medium containing either 0.3 mg/l BAP and 0.1 mg/l IAA or 2.5 mg/l TDZ only for two weeks and kept in darkness at 4°C (i.e., cold treatment) for another two weeks. Finally, callus cultures were transferred to solid MS medium containing either BAP (1.0 mg/l) and IAA (0.3 mg/l) or 2.5 mg/l TDZ only and incubated at 25°C under a 16 h light / 8 h dark regime for shoot development. Shoots were then readily rooted on solid MS medium containing 3.0 mg/l IBA only.

Statistical Analysis

Experiments were repeated 3 times using a complete randomized block design. Analysis of variance was carried out and differences between the means of the treatments were determined by Duncan's Multiple Range test at p 0.05 and 0.01. The analysis of variance and means were carried out with the SAS statistical program, using Proc ANOVA and Proc Mean procedures (SAS Institute, 1988).

Results

The effects of two different concentrations of BAP (0.1 and 0.25 mg/l) in combination with two different concentrations of 2,4-D (0.1 and 0.25 mg/l) on the growth of cell suspension cells of lines M114, ELK345, ÇBM315 and M1017 were examined. Results of analysis of variance for all treatments are summarized in Table 2. The effects of cytokinin, auxin and days of culture on the growth of suspension cells were found to be significant at p 0.01 level for all breeding lines. But the difference between cytokinin concentrations in terms of cell growth rate were not significant for the line M1017.

When callus was placed in liquid culture, white and friable callus was easiliy broken apart and dispersed into clumps of 0.5-5.0 mm. Further agitation fragmented these clumps into small cell aggregates. Freely suspended cells and cell clusters in a typical cell suspension culture of sugar beet are shown in Figure 1a. The growth patterns of suspension cells of each breeding line are presented separately below.

Growth Pattern of Suspension Cells of Line M114

The data indicates that adding 0.25 mg/l BAP and 0.25 mg/l 2,4-D to the culture medium increased the cell growth rate significantly in line M114, with a mean rate of 50.8% compared to 24.2% to 41.7% obtained in other treatments (Table 3). When the means of two BAP levels were compared, regardless of the concentrations of 2,4-D, it was found that 0.25 mg/l BAP produced significantly more cells (41.8%) than 0.1 mg/l BAP (33.0%). It was also clear that, when the level of BAP was ruled out, 0.25 mg/l 2,4-D was far more effective in increasing the number of cells in culture than 0.1 mg/l 2,4-D (46.3% and 28.5%, respectively). As shown in the

Table 2. Analysis of variance for cell growth (expressed as % PCV) of four breeding lines (see Tables 3-6 for further references).

		Mean Squares			
Source	DF	M114	ELK345	ÇBM315	M1017
Replication	2	65.4984	38.0978	25.3625	30.4729
Cytokinin	2	2504.0789 **	1030.3477 **	1994.1988 **	12.7115 NS
Auxin	2	3151.7829 **	2773.5905 **	1064.2978 **	214.9730 **
Time	7	9358.9063 **	4386.5015 **	3239.7670 **	949.2720 **
Error	106	88.5305	48.5058	30.6849	19.1904

**: Significant at p 0.01
NS: Not significant

Table 3. Effects of different concentrations and combinations of BAP and 2,4-D on the growth of cell suspension cultures (expressed as % PCV) in line M114. Means followed by the same letter are not significantly different at p 0.05 according to Duncan's Multiple Range test.

	BAP (mg/l)	0.0	0.1		0.25		
	2,4-D (mg/l)	0.0	0.1	0.25	0.1	0.25	Means
Days	0	3.2±0.1	3.2±0.1	3.6±0.3	3.5±0.3	4.2±0.2	3.6 g
	3	3.6 ±0.1	3.6±0.1	14.5±1.7	13.5±0.7	10.9±0.1	9.2 f
	5	11.5±0.5	11.4±0.3	23.0±0.6	15.0±0.2	16.8±0.1	15.5 e
	7	19.7±1.8	19.9±1.0	32.3±1.8	23.8±0.6	27.5±0.2	24.7 d
	9	29.6±1.9	29.1±3.0	44.3±4.7	30.9±0.5	73.9±0.9	41.6 c
	11	39.6±1.2	37.1±1.7	66.4±5.9	43.8±2.1	80.3±0.5	53.4 b
	13	43.6±1.3	44.2±1.5	73.6±2.4	57.2±0.1	96.1±0.4	62.9 a
	15	45.8±1.3	45.2±1.3	76.0±2.0	74.1±2.4	96.8±0.7	67.6 a
	Means	24.6 d	24.2 d	41.7 b	32.7 c	50.8 a	
Comparison of BAP Levels			33.0 b		41.8 a		
Comparison of 2,4-D Levels			28.5 b		46.3 a		

last column of Table 3, the growth rate of cells was slower at the beginning (9.2% at day 3), but at the end of culture period (day 15), a significantly higher biomass accumulation (67.6%) was recorded.

Growth Pattern of Suspension Cells of Line ELK345

A slightly different pattern was observed in this line. A level of 0.25 mg/l 2,4-D combined with either 0.1 or 0.25 mg/l BAP induced significantly more cell divisions than 0.1 mg/l 2,4-D, mean rates being 34.3% and 35.0% at 0.25 mg/l 2,4-D compared to 14.9% and 15.6% at 0.1 mg/l 2,4-D, respectively (Table 4). When the means of two BAP levels were compared regardless of the concentrations of 2,4-D, there was no significant difference between levels of BAP, with a mean of 24.6% at 0.1 mg/l BAP and 25.3% at 0.25 mg/l. However, it was clear that a higher concentration of 2,4-D was much more effective in cell division than the lower 2,4-D; 34.7% at 0.25 mg/l compared to 15.3% at 0.1 mg/l (Table 4). As shown in the last column of Table 4, the growth rate of cells was slower at the beginning (6.0% at day 3), but at the end of culture (day 15), a significantly higher biomass accumulation (47.0%) was achieved.

Growth Pattern of Suspension Cells of Line ÇBM315

The effect of BAP on cell division seemed to be more obvious than that of 2,4-D in line ÇBM315, which was the opposite in line ELK345. The 0.25 mg/l BAP caused a mean cell increase of 23.6% and 28.3% when combined with 0.1 or 0.25 mg/l 2,4-D, respectively (Table 5). However, it was low (about 16% on the average) when a lower BAP (0.1 mg/l) was combined with 2,4-D. This was further confirmed when the overall means of 0.1 and 0.25 mg/l BAP were taken into account as growth rates of cell suspension increased from 16.4% at 0.1 mg/l BAP to 26.0% at 0.25 mg/l BAP. The difference between the means of two levels of 2,4-D (19.8% and 22.5%) was significant and the effect of 2,4-D on cell division seemed to increase with increasing levels of BAP. Again, the growth rates of cells were slightly slower at the beginning (5.2% at day 3), but at the end of the culture period (day 15), a significantly higher biomass accumulation, reaching up to 43.6%, was observed (see the last column of Table 5).

Growth Pattern of Suspension Cells of Line M1017

The growth pattern of line M1017 was completely different from that of the others. In contrast to the other

Table 4. Effects of different concentrations and combinations of BAP and 2,4-D on the growth of cell suspension cultures (expressed as % PCV) in line ELK345. Means followed by the same letter are not significantly different at p 0.05 according to Duncan's Multiple Range test.

	BAP (mg/l)	0.0	0.1		0.25		
	2,4-D (mg/l)	0.0	0.1	0.25	0.1	0.25	Means
Days	0	1.3±0.4	2.6±0.5	3.2±0.2	3.2±0.2	3.1±0.1	2.7 g
	3	1.5±0.3	3.1±0.5	12.1±0.1	3.5±0.3	9.6±0.3	6.0 f
	5	6.0 ±2.1	6.8±2.9	14.1±0.2	7.1±2.8	14.1±0.2	9.6 e
	7	8.9±1.7	8.7±1.2	23.4±0.3	9.1±0.9	23.3±0.6	14.7 d
	9	15.1±1.8	13.0±0.9	40.7±1.0	14.8±0.6	44.0±0.2	25.5 c
	11	21.0±1.1	20.4±1.1	56.4±0.6	20.8±1.1	63.9±0.1	36.5 b
	13	27.5±0.6	27.9±0.8	59.6±0.7	28.9±0.8	61.8±0.9	41.1 b
	15	35.7±0.8	36.9±0.8	64.8±1.7	37.3±0.9	60.6±0.3	47.0 a
	Means	14.6 b	14.9 b	34.3 a	15.6 b	35.0 a	
Comparison of BAP Levels			24.6 a		25.3 a		
Comparison of 2,4-D Levels			15.3 b		34.7 a		

Table 5. Effects of different concentrations and combinations of BAP and 2,4-D on the growth of cell suspension cultures (expressed as % PCV) in line ÇBM315. Means followed by the same letter are not significantly different at p 0.05 according to Duncan's Multiple Range test.

	BAP (mg/l)	0.0	0.1		0.25		
	2,4-D (mg/l)	0.0	0.1	0.25	0.1	0.25	Means
Days	0	2.8±0.8	1.7±0.1	3.7±0.6	2.8±0.4	3.9±0.6	3.0 h
	3	3.1±0.8	2.0±0.0	8.5±0.3	3.3±0.2	9.0±0.4	5.2 g
	5	4.0±0.6	4.4±0.6	9.4±0.1	11.3±0.2	10.5±0.3	7.9 f
	7	5.9±0.9	7.7±1.9	13.3±0.3	19.3±0.7	18.6±0.7	13.0 e
	9	8.5±2.0	12.9±3.6	16.0±0.1	28.8±0.9	32.8±0.2	19.8 d
	11	12.8±2.4	21.4±5.7	20.5±0.4	35.0±1.2	45.5±0.3	27.1 c
	13	20.3±3.7	33.4±6.2	27.5±0.3	43.3±1.7	47.3±0.2	34.4 b
	15	34.8±3.2	43.7±3.4	35.5±0.3	45.1±1.1	58.7±0.3	43.6 a
	Means	11.5 d	15.9 c	16.8 c	23.6 b	28.3 a	
Comparison of BAP Levels			16.4 b		26.0 a		
Comparison of 2,4-D Levels			19.8 b		22.5 a		

three lines, cells of line M1017 divided more frequently at low (0.1 mg/l) 2,4-D than at high 2,4-D (0.25 mg/l); 16.3% and 10.5%, respectively (Table 6). There were no significant differences between BAP levels (12.9% and 13.9%). As expected, the growth rates of cells were slower at the beginning of culture (5.9% at day 3) but a significantly higher biomass accumulation, up to 25.6%, was recorded on day 15 (see the last column of Table 6).

Overall Comparisons of Growth Rates in Suspension Cells of All Lines

A prominent genotypic variation was evident when the means of all treatments (i.e., culture durations from 0 to 15 days, different combinations of 0.1 or 0.25 mg/l BAP and 0.1 or 0.25 mg/l 2,4-D) were calculated for each line (Table 7). Line M114 was, by far, the best responding

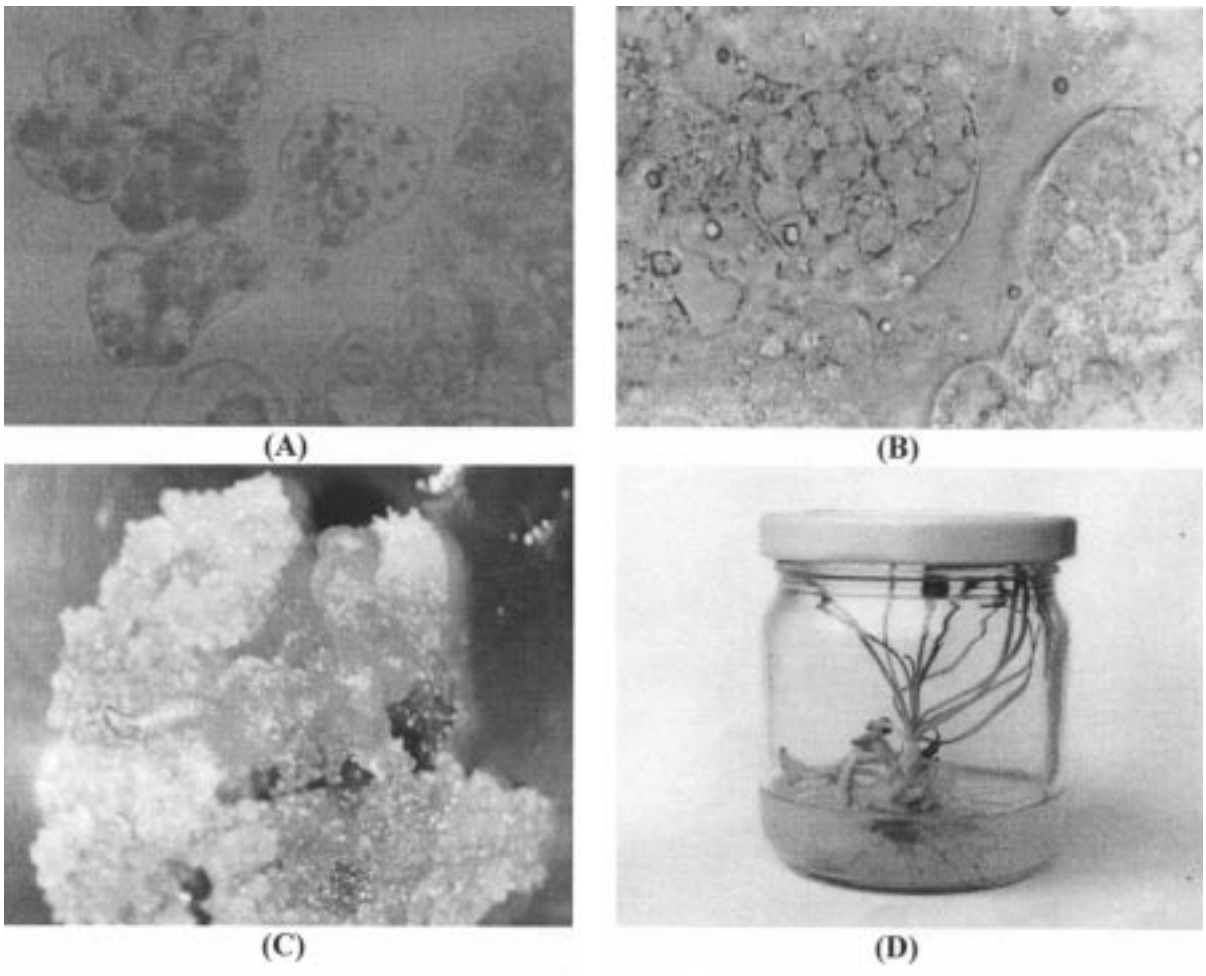

Figure 1. **A)** A light microscopy photograph showing released single cells and cell clusters derived from callus of line M114 when cultured in liquid MS medium containing 0.1 mg/l 2,4-D and 0.1 mg/l BAP (x2000). **B)** A spherical group of dividing cells in suspension observed on the 10th day of culture (x1000). **C)** A single shoot developed on the surface of suspension-derived callus of line ELK345 after culturing on solid MS medium containing 1.0 mg/l BAP and 0.3 mg/l IAA for 30 days (x0.5). **D)** A rooted shoot on MS medium containing 3.0 mg/l IBA.

genotype and the differences between the means of cell growth rates of the lines were statistically significant at p 0.05.

Shoot Differentiation from Suspension-Derived Callus

Active cell division was observed usually by 1-2 days of culture. Initially, cells were highly vacuolated, and then by day 5, morphologically distinct cells with a detectable cytoplasm and nucleus were visible. A spherical group of dividing cells was observed by 7-10 days (Figure 1b). By day 14-16, these cells divided further and formed undifferentiated cell colonies. The colonies were then recovered from the suspension and plated onto agar medium for further growth. By 27-30 days of culture, shoots appeared on the surface of the callus mass (Figure 1c). Approximately 50% of the suspension-derived callus gave rise to shoots when cultured on medium containing either 1.0 mg/l BAP combined with 0.3 mg/l IAA or 2.5 mg/l TDZ alone. The regenerated shoots were then readily rooted on medium containing 3.0 mg/l IBA (Figure 1d).

Discussion

During the incubation period, the biomass of suspension cultures increases due to cell division and cell enlargement. This continues for a limited period after

Table 6. Effects of different concentrations and combinations of BAP and 2,4-D on the growth of cell suspension cultures (expressed as % PCV) in line M1017. Means followed by the same letter are not significantly different at p 0.05 according to Duncan's Multiple Range test.

	BAP (mg/l)	0.0	0.1		0.25		
	2,4-D (mg/l)	0.0	0.1	0.25	0.1	0.25	Means
Days	0	3.0±0.3	3.5±0.5	4.3±0.1	4.3±0.2	4.4±0.1	3.9 f
	3	3.6±0.4	3.8±0.5	7.9±0.2	5.5±0.2	8.6±0.3	5.9 e
	5	5.2±0.8	6.6±0.6	9.4±0.1	9.5±0.2	8.8±0.1	7.8 d
	7	7.4±0.3	10.5±1.0	10.4±0.3	12.4±0.3	9.5±0.1	10.2 d
	9	14.3±2.6	14.8±1.7	12.0±0.1	18.0±0.1	10.2±0.2	13.7 c
	11	20.7±2.9	20.3±1.2	12.3±0.3	22.3±0.2	11.5±0.5	17.5 b
	13	25.3±3.4	29.1±0.6	13.0±0.5	31.8±0.1	15.0±1.2	22.7 a
	15	28.6±3.2	34.8±2.7	13.6±0.7	34.5±0.0	16.4±0.9	25.6 a
	Means	13.5 b	15.4 ab	10.4 c	17.2 a	10.5 c	
Comparison of BAP Levels			12.9 a		13.9 a		
Comparison of 2,4-D Levels			16.3 a		10.5 b		

Table 7. An overall comparison of the lines in terms of the mean cell growth rates measured by PCV method after 15 days of culture in liquid medium in the dark. Means followed by the same letter are not significantly different at p 0.05 according to Duncan's Multiple Range test.

Lines	Mean Cell Growth Rate (%)
M114	34.8 a
ELK345	21.5 b
ÇBM315	19.2 c
M1017	13.4 d

which the growth stops due to the exhaustion of some factors or the accumulation of certain toxic metabolites in the culture medium. If at this stage a small aliquot of the cell suspension with uniformly dispersed cells and cell aggregates is transferred to a fresh medium of the same composition (i.e., subculture), cell growth is revived.

In our cultures, the growth rates of cells in suspension were rather slow at the beginning of the culture (day 3), ranging from a mean of 5.2% to 9.2% PCV in different lines, but at the end of 15 days' culture a significantly higher biomass accumulation was achieved, with means ranging from 25.6% to 67.6%. With the dilution rate of 30/50 during the subcultures, a stationary phase of cell growth was reached after nearly one week in the experiments conducted in this study. This is consistent with the findings of Van Geyt & Jacobs (1985) and De Greef & Jacobs (1979). For use in our following experiments on protoplast isolation, suspension cultures were maintained for more than two months by subculturing at two-day intervals since it is known that prolonged maintenance of cultures in the stationary phase could result in the extensive death or lysis of the cells (Bhojwani & Razdan, 1990). However, cultures can be maintained continously in the exponential phase by frequent subcultures (at every 2-3 days) of the suspensions.

To obtain a fine suspension culture, it is of prime importance to initiate suspension cultures from a friable callus source. Therefore, white and friable callus (Type I) obtained from our callus culture experiments (Gürel et al., 2001) were used. The Type I callus contained large and translucent cells and formed a much more friable structure than the small-celled compact tissue of green callus (Type II). As the friability of the cells increases, it becomes much easier to achieve a full separation of the cells. It was also reported that the degree of the friability of the callus tissue increased when maintained on a semi-solid medium for two to three passages (Bhojwani & Razdan, 1990).

Plant cell cultures are normally established and maintained on media containing an auxin and a cytokinin. Removal of either hormone from the medium would normally result in culture death (Stafford, 1996). We obtained higher rates of cell growth when the cells were

cultured in a liquid medium containing BAP and 2,4-D whereas much lower rates of cell division occurred when the culture medium contained no plant growth regulators (i.e., hormone-free medium). However, the optimal concentration and combination of 2,4-D and BAP varied greatly from line to line, indicating a role of genotypic variation in the success of cultures. Usually, a combination of high auxin/high cytokinin induced more cell divisions but the critical role of the type of plant growth regulators differed among the lines. The auxin 2,4-D was the limiting factor for cell division in lines M114, ELK345 and ÇBM315, whereas it was the cytokinin BAP in line M1017. This finding is a further support for genotypic variation frequently observed in tisssue cultures of sugar beet (Doctrinal et al., 1989; Gürel, 1997). When the means of all treatments were taken into account, a greater variation among the lines was evident, with lines M114, ELK345, ÇBM315 and M1017 producing a mean cell growth rate of 34.8%, 21.5%, 19.2% and 13.4%, respectively.

Shoots were readily obtained, in all lines, from callus derived from cell suspension cultures when the callus was pre-treated with cold at 4°C for two weeks in darkness followed by incubation on medium containing BAP in combination with IAA or on medium containing TDZ alone. The important role of cytokinins (especially BAP) and TDZ on shoot differentiation from callus or directly from the explant tissue of sugar beet has also been reported by others (Coumans et al., 1982; Saunders, 1982; Roussy et al., 1996).

Sugar beet is an economically important crop in Turkey but, unfortunately, not much progress has been made to improve its agronomic traits. In this sense, the genetic improvement of this species through biotechnological approaches becomes more significant. This report describes the establishment of suspension culture for several breeding lines of sugar beet. We aim to obtain protoplasts from these suspensions for the production of somatic hybrids.

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