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In vitro multiplication of *Beta vulgaris* L. throughout excised shoot tips

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Abstract

The excised shoot tips of *Beta vulgaris* L. cvs. Ras poly, Gala and Lola were inoculated aseptically on Murashige and Skoog's (MS) medium with 0.25 mg dm^{-3} α-naphthaleneacetic acid (NAA) and 1.00 mg dm^{-3} 6-benzylaminopurine (BAP). They produced multiple shoots at relatively high frequency. For shoot multiplication, a concentration of 1.00 mg dm^{-3} NAA and 0.25 mg dm^{-3} BA in the culture medium was found to be optimum for all the cultivars. MS medium supplemented with 3.0 mg dm^{-3} 3-indolebutyric acid (IBA) and 0.02 mg dm^{-3} N-isopentenylaminopurine (2ip) was found most effective for rooting of micropropagated shoots. *In vitro* produced plantlets were successfully transferred to the soil. The percentage of survival was more than 95 % and micropropagated plants showed no morphological differences from those grown naturally.

Additional key words: growth regulators, micropropagation, sugar beet.

Introduction

Tissue and cell cultures techniques provide many new opportunities for improving the genetic and breeding work of sugar beet, *Beta vulgaris* L. Differentiation of sugar beet plants from callus and suspension cultures has been reported by several researchers (Atanassov 1986, Butenko *et al.* 1972, Enomoto and Ohyama 1985, Doley and Saunders 1989, Yurkova *et al.* 1990, Owens and Eberts 1992, Potyondi 1993). Also the *in vitro* vegetative propagation of sugar beet has been studied rarely. Many different types of tissue have been used as explants such as excised axillary buds (Mezei *et al.*1990, Mezei and Kovacev 1991), growing points of peduncle (Goska and Rogozinska 1990), apical meristems (Goska and Szota 1992), flower buds (Seilova and Amerkhanova 1990), excised shoots (Sullivan *et al.* 1993), and from inflorescence pieces (Zhong *et al.* 1993).

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Abbreviations: BAP - 6-benzylaminopurine; IBA - 3-indolebutyric acid; 2iP - N-isopentenylaminopurine; NAA - α-naphthaleneacetic acid.
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The aim of this study was to characterize the conditions for *in vitro* shoot initiation, shoot multiplication and rooting of three cultivars of sugar beet *Beta vulgaris* L.

Materials and methods

Plants: The sugar beet (*Beta vulgaris* L. ssp. *vulgaris* var. *altissima*) cvs. Ras poly, Gala and Lola were used, kindly provided by Sugar Crops Research Institute of Agriculture Research Centre, Giza, Egypt.

Preparation of explant, culture media and culture conditions: Sugar beet seeds were washed with distilled water and then immersed in 70 % ethanol for 2 min and transferred to a solution of 30 % commercial *Clorox* (containing 5.25 % sodium hypochlorite) for 25 min, then finally washed four times with distilled sterilized water. Seeds were soaked in distilled sterilized water overnight. For obtaining seedlings, five seeds were placed in 250 cm^3 flasks containing 50 cm^3 MS basal medium (Murashige and Skoog 1962) without any supplementation of growth regulators. Shoot tips were obtained from aseptically grown sugar beet seedlings (15-d-old), and cultured on MS medium supplemented with different concentrations of BAP or of NAA and BAP. For shoot multiplication, the following media were tested:

M1 - MS + 0.50 mg dm^{-3} BAP
M2 - MS + 0.25 mg dm^{-3} NAA + 1.00 mg dm^{-3} BAP
M3 - MS + 0.50 mg dm^{-3} NAA + 0.50 mg dm^{-3} BAP
M4 - MS + 1.00 mg dm^{-3} NAA + 0.25 mg dm^{-3} BAP

For rooting the following media were used:

R1 - MS basal medium
R2 - MS + 1.00 mg dm^{-3} IBA
R3 - MS + 2.00 mg dm^{-3} IBA
R4 - MS + 3.00 mg dm^{-3} IBA
R5 - MS + 3.00 mg dm^{-3} IBA + 0.02 mg dm^{-3} 2ip

The pH of the media was adjusted to 5.8 using 1 M of either NaOH or HCl, then autoclaved at 121 °C and a pressure of 1.2 kg cm^{-2} for 20 min. Cultures were incubated in a growth chamber at 25 ± 2 °C under a 16-h photoperiod (irradiance of 25 μmol m^{-2} s^{-1} provided by cool white fluorescent lamps). To evaluate media efficiency the following parameters were used: number of viable shoots formed per explant and shoot length, multiplication rate (average number of shoots per explant), and number of roots and root length.

Results and discussion

Establishment of cultures: With a view to optimizing the conditions for shoot initiation and proliferation from shoot tips of different cultivars of sugar beet the

effect of BAP added alone or in combination with NAA in the culture media were investigated. It was observed that the presence of BAP alone or in combination with NAA in the culture media induced shoot proliferation with all cultivars (Fig. 1). Response of the three cultivars were different according to the concentrations of growth regulators in the shoot inducing media. The response was lowest when the culture media contained BAP alone.

The highest number of shoots occurred when shoot tips were grown on MS medium with 0.25 mg dm^{-3} NAA and 1.00 mg dm^{-3} BA. Among the three cultivars tested, cv. Gala showed the highest number of shoots differentiated from a shoot tip explant (8.4), followed by cvs. Lola and Ras poly (7.6 and 5.4, respectively). The highest shoot length was at the same combination of NAA and BAP, while no

Table 1. Effect of different concentrations of BAP alone or in combination with NAA [mg dm^{-3}] in MS culture medium on number of shoots and shoot length of different cultivars of *Beta vulgaris* L. recorded 30 d after culture (5 explants per treatment; mean ± SD).

Growth regulators		Number of shoots per explant			Shoot length [cm]		
NAA	BAP	Ras poly	Lola	Gala	Ras poly	Lola	Gala
0.0	0.25	3.8±0.83	5.4±0.54	7.2±0.83	1.30±0.21	3.00±0.07	3.40±0.15
0.0	0.50	3.6±0.54	6.2±0.83	6.8±0.83	1.22±0.19	3.22±0.19	3.24±0.20
0.0	1.00	4.4±0.54	5.4±0.54	6.4±0.54	1.12±0.14	2.74±0.18	3.24±0.20
0.0	2.00	3.8±0.83	5.4±0.54	6.0±0.70	1.00±0.14	2.66±0.25	3.06±0.11
0.25	0.25	4.6±0.54	6.2±0.83	8.4±0.89	1.28±0.19	3.28±0.19	3.16±0.20
0.25	0.50	4.6±0.50	5.6±0.89	7.4±0.54	1.38±0.13	3.76±0.11	3.04±0.16
0.25	1.00	5.4±0.50	7.6±1.14	8.4±0.54	1.68±0.23	3.98±0.08	3.42±0.13
0.25	2.00	4.6±0.66	6.0±0.70	7.4±0.54	1.22±0.19	3.94±0.11	3.28±0.13

difference was observed between the single treatments with BAP (Table 1). The highest shoot length was achieved with cvs. Lola and Gala (3.98 and 3.42 cm, respectively).

An efficient micropropagation system for sugar beet was established by several investigators and different explants were used. The growth regulators was important for shoot proliferation. In this connection, it should be mentioned that Seilova and Amerkhanova (1990) showed that plantlets were formed from flower buds of a diploid sugar beet cultivar when cultured on MS medium with 2.0 mg dm^{-3} BAP. Mezei *et al.* (1990) found that explants taken from three genetically divergent genotypes of sugar beet, at ontogenetic phases earlier than the end of floral development produced more buds than those taken at full flowering, the inflorescence tip produced the greatest number of buds. Best micropropagation was achieved on a medium containing 0.50 mg dm^{-3} BAP. Goska and Szota (1993) stated that shoot proliferation were induced from apical meristems of different types of sugar beet when cultured on MS medium supplemented with 0.09 mg dm^{-3} NAA and 0.22 mg dm^{-3} BAP.

Shoot multiplication: As the best results were obtained in establishment stage on MS medium supplemented with 0.25 mg dm^{-3} NAA and 1.0 mg dm^{-3} BAP, the further

Fig. 1. Shoot differentiation from cultured shoot tips of sugar beet cultivars (form the left to the right: Gala, Ras poly, Lola, Gala) cultured on MS medium supplemented with 0.25 mg dm^{-3} NAA and 1.0 mg dm^{-3}

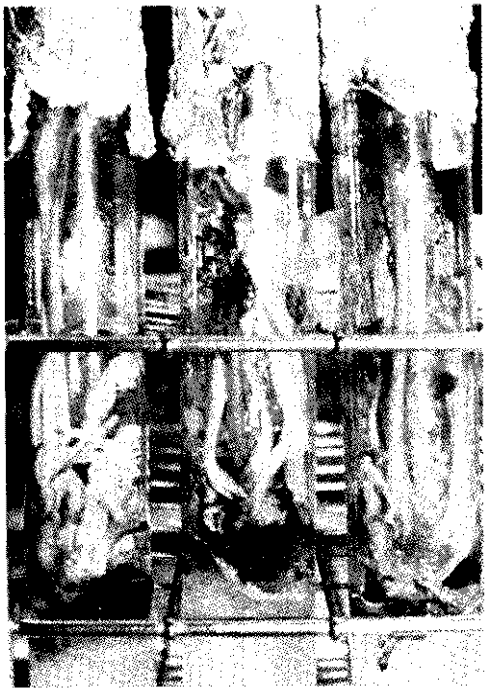

Fig. 3. Development of multiple shoot cultures of sugar beet cultivars, cultured on MS medium supplemented with 1.0 mg dm^{-3} NAA and 0.25 mg dm^{-3} BAP.

analyses of shoot multiplication and rooting were only with shoots grown on that medium. After 8-week cultivation on multiplication media (Fig. 2) the rate of BAP multiplication varied with the different sugar beet cultivars as well as the composition of multiplication medium. The lowest multiplication was on MS medium with 0.50 mg dm^{-3} BAP, on the contrary highest multiplication rate of all the cultivars was on MS medium with the lowest concentration of BAP (0.25 mg dm^{-3}). The highest rate of multiplication was observed with cv. Lola followed by cv. Gala and cv. Ras poly.

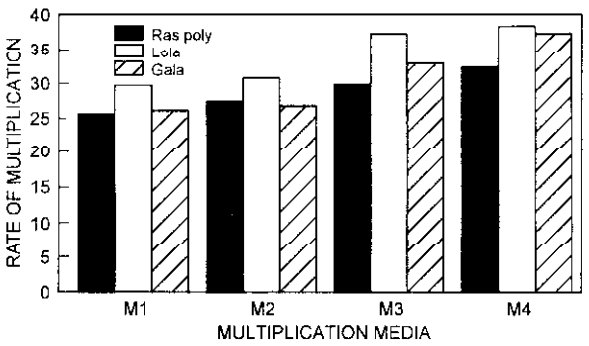

Fig. 2. Rate of multiplication of different cultivars of sugar beet after 8-week cultivation on different multiplication media.

In general, the addition of NAA (1.00 mg dm^{-3}) in combination with BAP (0.25 mg dm^{-3}) was found the optimal for multiplication rate with all the cultivars tested (Fig. 3). On this medium, elongated shoots of the different cultivars tested, rooted at different rates.

Goska and Rogozinska (1990) found that the optimum concentration of growth regulators was 1.0 mg dm^{-3} IBA and 0.20 mg dm^{-3} BAP for multiplication of shoots produced from growing points of the peduncle of various sugar beet genotypes. Results obtained are similar to those of Goska and Szota (1992) who mentioned that NAA (1.0 mg dm^{-3}) and BAP (0.22 mg dm^{-3}) added to MS medium resulted in high frequency of shoot multiplication of different types of sugar beet trisomics cultured in *in vitro*.

Rooting: After 4-week cultivation on basal MS medium (without growth regulators) the roots were induced. Addition of IBA (1.0 mg dm^{-3}) to the rooting medium showed no promoting effect on rooting. Shoots of cvs. Ras poly and Lola even failed to produce roots on medium containing 2.0 mg dm^{-3} IBA. The highest number of root was obtained when both IBA and 2ip were incorporated into the medium, where cv. Lola gave the higher number than the other cultivars (Table 2). The highest root length was obtained on MS medium supplemented with 3.0 mg dm^{-3} IBA and 0.02 mg dm^{-3} 2ip and cv. Lola showed the highest root length followed by cvs. Gala

and Ras poly (3.46, 3.20 and 2.86 cm, respectively). The *in vitro* production of plantlets with roots was very important for transferring of plantlets to soil (Fig. 4).

Some reports indicated that MS medium with 2.0 mg dm^{-3} IBA was the best for root development of sugar beet (Goska and Rogozinska 1990, Seilova and Amerkhanova 1990). Mezei *et al.* (1990) stated that best rooting for sugar beet plants produced *in vitro* was achieved on an auxin-free medium. Application of cold stress and red light on root induction in the *in vitro* micropropagation of various genotypes of sugar beet, resulted in an increased frequency of root formation (Melzer 1991). Recently, Zhong *et al.* (1993) found that MS medium supplemented with 1.0 mg dm^{-3} NAA and 3 % sucrose was the optimum for *in vitro* rooting of wild beet, *Beta maritima.* Results outlined above are in agreement with those reported by Goska and Szota (1992) as they found that the presence of 2ip (0.02 mg dm^{-3}) and IBA (3.0 mg dm^{-3}) in rooting medium promoted the formation of roots of nine types of sugar beet trisomics.

Table 2. Effect of different concentrations of IBA and 2ip [mg dm^{-3}] in root-inducing media on root number and length [cm] in proliferated shoots of different cultivars of *Beta vulgaris* L. after 30 d in culture (means + SD)

Growth regulators		Ras poly		Lola		Gala	
IBA	2ip	number	length	number	length	number	length
0.0	0.0	2.6±0.5	0.90±0.10	3.0±0.5	1.24±0.15	2.8±0.4	1.10±0.15
1.0	0.0	-	-	-	-	-	-
2.0	0.0	-	-	-	-	2.2±0.8	0.96±0.20
3.0	0.0	2.8±0.4	2.86±0.11	3.8±0.4	3.46±0.11	3.0±0.7	3.20±0.15
3.0	0.02	3.0±0.7	2.80±0.15	4.2±0.4	3.46±0.11	3.4±0.5	3.10±0.15

Transfer of plantlets to soil: *In vitro* produced sugar beet plants were thoroughly washed in tap water to remove agar from roots. Transplanting was done in pots using a 1:1 mixture of sand and peatmoss. Plastic bags covers were used to ensure high humidity around plants and pots were kept at 20 ± 2 °C with 16-h photoperiod (irradiance of 25 μmol m^{-2} s^{-1}). Plants were irrigated three times weekly with tap water. After two weeks covers were removed and plants were acclimatized for other two weeks (Fig. 5). Plants were easily established in soil. Survival of sugar beet plantlets was recorded after 4 weeks and was in average 95.5 % (93.3 % in cv. Ras poly and 96.6 % in cvs. Lola and Gala.

Regenerated plants showed no morphological differences from those grown naturally. The use of multiple clone lines, each one coming from one seed, enable the maintenance of variability through the micropropagation.

In this respect, Mezei *et al.* (1990) confirmed that *in vitro* vegetative propagation of sugar beets from floral explants is feasible. The genotypes multiplied from various explants were identical to the original material, indicating that the method could be useful in a breeding programme. Also, Zhong *et al.* (1993) reported that plantlets

produced from *in vitro* culture of inflorescence pieces of wild beet, *Beta maritima* were transferred to soil and placed in greenhouse showed no somaclonal variation.

Fig. 4. Rooted plantlets of sugar beet cultivars used after 4 weeks on MS medium supplemented with 3.0 mg dm^{-3} IBA and 0.02 mg dm^{-3} 2ip.

Fig. 5. Sugar beet plants, obtained *in vitro*, 2 weeks after transfer to a peat moss and sand mixture.

In conclusion, this study was conducted to evaluate the potential for *in vitro* propagation in diverse sugar beet cultivars grown in Egypt. The results confirmed that *in vitro* vegetative propagation from excised shoot tips of the tested cultivars is feasible in high frequency, the *in vitro* produced plants were identical to the *in vivo* plants, indicating that the method could be useful in breeding programmes.

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