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Indirect Regeneration Tissue Culture and Molecular Characterization for Some Sugar Beet (*Beta vulgaris* L.) Genotypes

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ABSTRACT

Sugar beet seeds are imported from European countries with expensive cost as well as many tries were procedured for flowering and seeds production but without any outcome, therefore that the use of tissue culture technique is a useful tool in Egypt. The investigation was carried out during 2017- 2018 at the laboratories of the cell research department, field crops research institute, agricultural research center, An indirect regeneration protocol for micropropagation of some sugar beet (Beta vulgaris L.) genotypes and the genetic variation were described. For callus production, hypocotyl, cotyledon explants were cultured on Murashige and Skoog's medium supplemented with 0.1 mg/l BAP+2.0 mg/l 2,4-D and cotyledon explants produced significantly more calli than hypocotyl explants. Significant difference among the genotypes and among the different media was observed. For shoot induction callus of different explants was transferred to MS-medium containing 1.0 mg/l BAP+0.2 mg/l IAA. The regenerated shoots were transferred to rooting MS-medium containing 3.0 mg/l IBA+0.3 mg/l NAA. The genetic variation among the sugar beet genotypes was based on RAPD- PCR data suggest a relatively high genetic diversity among them, that can be used in the breeding programs taking into account select the parents, which have the wide genetic distance.

***Keywords*: Sugar beet, seedling explants, callus induction, indirect shoots regeneration, molecular analysis and RAPD**

Introduction

Egyptian Government imports about 2250 tons of sugar beet seeds, every year to planting about 550 fed (fed = 0.42 ha). This is coasting about 9 thousand Euros per ton with total cost 21.100 million Euros. Sugar beet (Beta vulgaris L.) was introduced to Egypt in 1982 to overlap the vast gap between sugar consumption and production, which reached 57.7% (about 1.25 million ton sugar) of local needs of white sugar in 2019, Sugar beet represents the first main sugar crop for sugar production. In Egypt, sugar beet seeds imported because its default to produce seeds under Egypt weather conditions, It is led to many tries to produce seeds but without significant impact. In this paper, we tried to use the tissue culture tool for micropropagation of sugar beet. Sugar beet is considered one of important winter sugar crop in Egypt. So, it is preferable to evaluate sugar beet verities under Egyptian conditions to select the best ones characterized by high yield and quality traits to decrease the Egyptian gap from sugar.

Tissue culture techniques being developed and integrated with conventional breeding programs are playing a significant role in the agricultural improvement of sugar beet. The application of tissue culture technique is as an efficient method of asexual propagation Hu and Wang, (1983). Micropropagation has been considered as a useful technique for the improvement of sugar beet (Atanasov, 1980 and Mezei *et al*., 2006). Several studies are being carried out world-wide on the in vitro regeneration of sugar beet. Indirect regeneration of sugar beet has been reported by a number of workers (Slavova, 1981; Szabados, and Gaggero, 1985; Saunders, and Shin, 1986; Yu, 1989; Konwar, and Coutts, 1990 and Gurel *et al.* 2001).

In the most cases sugar beet can be multiplied *in vitro* by micro propagation or somatic organogenesis. Indirect organogenesis was obtained on calli deriving from sugar beet root, hypocotyl (Jacq *et al*., 1992), cotyledon (Jacq *et al*., 1993) and leaf (Yu, 1989). Regardless of starting explant

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and regeneration protocol used, this type of sugar beet regeneration and multiplication is based on interaction of genotype and concentrations of phytohormones in nutrient medium. Abundant clonal materials of single genotype could be very useful for breeding purposes as well as for physiological and pathological studies.

Despite recent achievements and advances in *in vitro* tissue culture of numerous plants, for sugar beet it is not very productive (Gurel and Gurel, 2013). Due to the variations, different genotypes responses to the same method are diverse (Pazuki *et al*., 2018). Therefore, one cannot always propagate the desired sugar beet genotypes sufficiently and efficiently. Micropropagation has been used for sugar beet cloning (Klimek-Chodacka and Baranski, 2013). For instance, protoplast fusion (Gurel *et al*., 2002), chromosome doubling, and even transformant regeneration produced after performing cutting-edge research (Karimi *et al*., 2015) may take advantage of follow-up micro propagation. Molecular markers find the importance of analysis in the differentiation between genotypes. Hallden *et al*. (1994) reported that RAPD markers are easier and quicker to use, these markers may be preferred in applications where the relationships between closely related breeding lines are of interest. The main objectives of this study was establishment a tissue culture protocol of sugar beet and determine the best explants for micropropagation, as well as, study the genetic variation and knowledge of the diversity and similarity genetic among some sugar beet genotypes.

Materials and Methods

The experiment was conducted during 2017- 2018 at the laboratories of Cell Res. Dept. (CRD), Field Crops Res. Institute (FCRI), Agric. Res. Center (ARC), Giza, Egypt and Greenhouse Horticulture Dept, Fac. of Agric. Ain Shams Univ. to establishment a plant regeneration protocol and genetic characterization for ten genotypes of sugar beet (*Beta vulgaris* L.).

Plant material

Sugar beet seeds of ten (Multi & Monogerm) genotypes as shown in Table (1) from Sugar Crops Institute (SCI)*, ARC, Giza, Egypt.

Table 1: Sugar beet genotypes used in this study.

Number	Name	Type
1	FD 0719	Multigerm
2	Despre 2	Multigerm
3	Tenor	Multigerm
4	Bts 301	Multigerm
5	Bts 237	Multigerm
6	Franando	Monogerm
7	Toucan	Monogerm
8	Sibel	Monogerm
9	Univers	Monogerm
10	LLP Ⅲ	Monogerm

*Seeds were imported from Germany

1. Establishment of sugar beet regeneration protocol

Ten sugar beet genotypes Table (1) were used for establishing a regeneration protocol. Regeneration of plantlets was via adventitious buds derived from callus. Seeds were surface sterilized by dipping in70% (v/v) ethanol for 5 min, followed by immersion in 7.5% (v/v) sodium hypochlorite for one hour in the presence of 0.5 ml Tween 20 per 100 ml solution, then rinsed several times with sterile distilled water and left in sterile distilled water for 16-20 hours. After sterilization, seeds were blot dried on sterilized Whatman filter paper and were cultured on MS medium (Murashige & Skoog, 1962) containing 3% (w/v) sucrose as carbon source, 0.8% (w/v) agar as solidified agent.

Hypocotyl and cotyledon explants were taken from 10-12 day-old seedlings and cultured for callus formation on different MS-medium supplied by 0.5 mg/l BAP (6-Benzilaminopurine) + 1.0 mg/l 2,4-D (2,4-Dichlorophenoxy acetic acid), 0.1 mg/l BAP+ 2.0 mg/l 2,4-D and 1.5 mg/l BAP+ 3.0 mg/l 2,4-D.

For shoot induction, derived calli were culture on different half-strength MS-Medium supplied by 0.5 mg/l BAP+0.1 mg/l IAA (Indole-3-aceticacid), 0.1 mg/l BAP+0.2 mg/l IAA and 1.5 mg/l BAP+0.3 mg/l IAA. Derived shoots were culture on half-strength MS-Medium supplemented with 1.0 mg/l IBA (Indolebutyric acid) + 0.1 mg/l NAA (1-Naphthalene acetic acid), 2.0 mg/l IBA+ 0.2 mg/l NAA and 3.0 mg/l IBA with 0.3 mg/l NAA for root formation. All used media were adjusted to 5.8with 1M KOH or HCland autoclaved at 121°C and 1.2 kg.cm^2 for 20 minutes. After culture, medium was incubated at 25°C under a 16/8 hr's light/dark photoperiodic regime (1000-Lux) and 70% humidity.

Callus formation was estimated after 4-6 weeks, while shoot and root formation was estimated after 8 weeks and the resulting plantlets were transferred to greenhouse for acclimatization.

Experimental design

The experiment was carried out in a completely randomized design and was subjected to two-factor of analysis of variance with ten replicates. The results were expressed as the percentage of explants producing calli, the percentage of adventitious shoot induced from calli and the percentage of plantlets induced from shoots.

Molecular analysis

Genomic DNA was extracted from germinated seeds usinga CTAB protocol (Torres *et al*., 1993). DNA quality was checked using agarose gel electrophoresis. DNA qualityand quantity were determined by spectrophotometer (Thermo Scientific, Genesys 10 S UV-Vis) and samples were diluted to a final concentration of 50 ng/µL.

PCR analysis

A total of 12 RAPD primers were used (Integrated DNA Technologies) as shown in Table (2). PCR reactions with RAPD primers were carried out in 25-µLreactions containing 2 µL (50 ng) of genomic DNA, 2.5µL of PCR buffer (10X) containing $MgCl_2$ (15 mM), 1µLof 10 mM dNTPs, 0.2 µL of Taq DNA polymerase (3 U/µL), and 2 µL (15 ng) of each primer. PCR with RAPD primers was performed in a T100 thermal cycler (Bio-Rad) as follows: predenaturation at 94 °C for 4 min; 35cycles of denaturation at 94 °C for 1 min, annealing at 35°C for 1 min, and extension at 72 °C for 3 min; and final extension at 72 °C for 0 min.

Table 2. List of used RAPD primer names and their nucleotide sequences.

Primer names	Sequence `5→`3
R1	GGTGCGGGAA
R2	GTTTCGCTCC
AN-1	ACTCCACGTC
AH-5	TTGGAGGCAG
Z-4	AGGGTGTGCT
Z-10	CCGACAAAGC
D-6	ACCCGGTCAC
D-10	GGTCATCACC
D-12	CACCGTATCC
O-2	TCGGCGGTTC
C-20	ACTTCGCCAG
BA-5	GGACGTTGAG

Statistical Analysis

Data were subjected to analysis of variance (ANOVA), and the means were compared using Duncan multiple range test at 0.05 confidence level by the use of MSTAT-C computer programs Waller and Duncan (1969).

Amplified fragments were presented in a matrix form of binary data, in which the 'presence' or 'absence' of PCR fragments with the same molecular weight was scored as 1or 0, respectively. Cluster analysis was performed using the unweight pair-group method with arithmetical average (UPGMA) and dendrograms were constructed on the basis of Nei's genetic distance index (1978) for

RAPD. To assess the validity of the correlation between the matrices, the Mantel (1967) test was used. Statistical analysis was performed using Power Marker (Liu and Muse, 2005) and SPSS (2003) software packages.

Results and Discussion

1-Establishment of sugar beet regeneration protocol

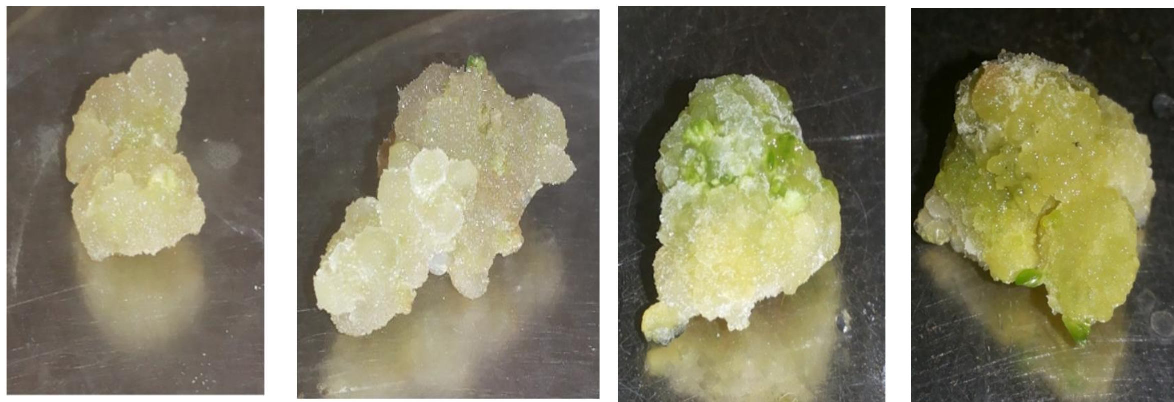
After germination, 10-12 days old, cotyledon and hypocotyls explants of ten sugar beet genotypes were cultured. Callus formation was estimated after 4-6 weeks from culture. Only three genotypes succeeded in producing callus FD 0719, Toucan and Univers were ranked 1, 7 and 9, respectively in (Table 3).

Data in (Table 3)indicate that there were significant differences among different media and genotypes and their interaction.The best callus induction percentage was achieved by the supplementation of 0.1 mg/l BAP+2.0 mg/l 2,4-D medium. Toucan genotype gave maximum callus induction percentage (66.11%).The highest value of callus frequency percentage was observed by culturing Univers and Toucan genotypes with0.1 mg/l BAP+2.0 mg/l 2,4-D medium (76.00% and 75.33%),respectively, followed by FD 0719genotype (68.00%),(Fig. 1).

Table 3: Effect of cotyledon explant and different media on mean callus induction percentage of three sugar beet genotypes

Media mg/l	Genotypes			Mean
	FD 0719	Toucan	Univers	
0.5 mg/l BAP + 1.0 mg/l 2,4-D	56.00^{cd}	60.00^{bcd}	51.33^{cd}	**55.78^{B}**
0.1 mg/l BAP + 2.0mg/l2,4-D	68.00^{ab}	75.33^{a}	76.00^{a}	**73.11^{A}**
1.5 mg/l BAP + 3.0mg/l2,4-D	57.33^{bcd}	63.00^{bc}	49.33^{d}	**56.55^{B}**
Mean	**60.44^{AB}**	**66.11^{A}**	**58.89^{B}**	

Means with the same letter within each row or column are not significantly different at p=0.05 according to Duncan's Multiple Range Test.



(A) **(B)** **(C)** **(D)**

Fig. 1: White and friable callus derived from hypocotyl explant (A), (B) and green and compact callus derived from cotyledon explant (C), (D) cultured on MS-medium containing 0.1 mg/l BAP + 2.0 mg/l 2,4-D for Toucan genotype after 6 weeks.

Respecting the percentage of the initiated and formed callus from hypocotyl explants, data in (Table 4) showed that there were significant differences among studied media and the highest percentage of the formed callus (32.89%) for MS-medium supplemented with 0.1 mg/l BAP+2.0 mg/l 2, 4-D, whereas, MS-medium supplemented with 0.5 mg/l BAP+1.0 mg/l 2, 4-D produced the lowest percentage of callus formation (27.11%).

Data in (Table 4) also showed slightly significantly differences among the three genotypes. Toucan genotype gave the highest percentage value of initiated calli (32.22%), whereas, Univers genotype recorded lowest percentage value (26.00%) and the interaction between studied media and

genotypes no significantly effect. It was cleared from (Tables3&4) that the cotyledon explants gave higher values and better quality of callus than the hypocotyl explants for all studied media and genotypes, (Fig. 1).

Table 4. Effect of hypocotyl explant and different media on mean callus induction percentage of three sugar beet genotypes

Media mg/l	Genotypes			Mean
	FD 0719	Toucan	Univers	
0.5 mg/l BAP + 1.0 mg/l 2,4-D	30.00^{bc}	29.33^{bc}	22.00^{c}	**27.11^{B}**
0.1 mg/l BAP + 2.0 mg/l 2,4-D	31.33^{ab}	38.00^{a}	29.33^{bc}	**32.89^{A}**
1.5 mg/l BAP + 3.0 mg/l 2,4-D	30.67^{ab}	29.33^{bc}	26.00^{bc}	**28.67^{AB}**
Mean	**30.67^{A}**	**32.22^{A}**	**26.00^{B}**	

Means with the same letter within each row or column are not significantly different at p=0.05 according to Duncan's Multiple Range Test.

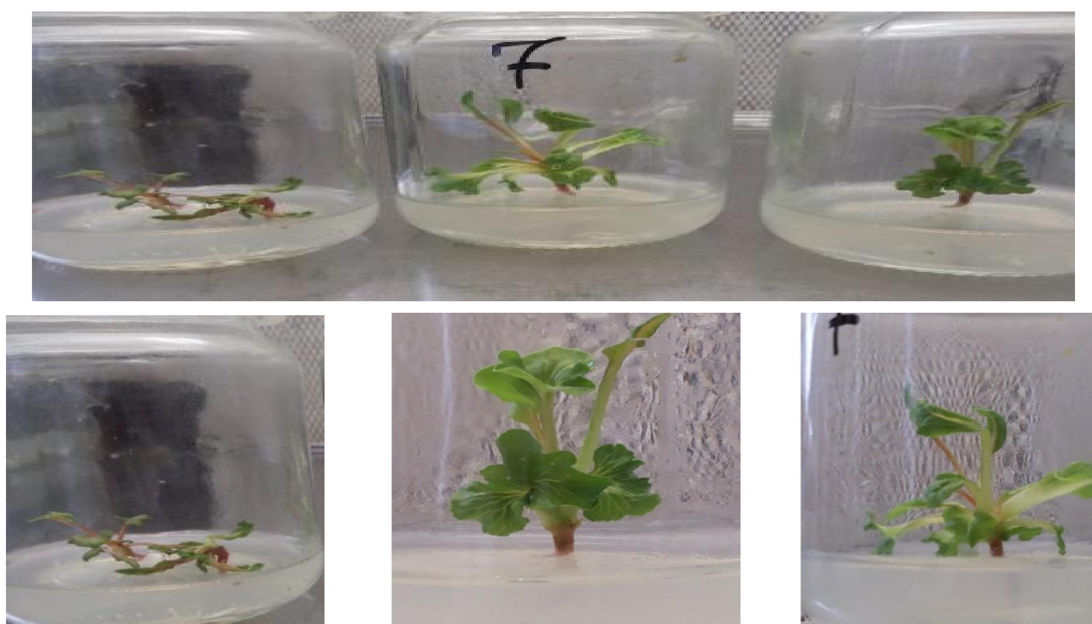


Fig. 2: Shoots developed on half-strength MS-medium containing 1.0 mg/l BAP + 0.2 mg/l IAA after eight weeks for Toucan genotype.

The regeneration frequencies of the cotyledon derived calli from three sugar beetgenotypes are listed in (Table 5). It was signifcant differences among studied medium as well as among studied genotypes.Data indicated that the best regenerated shoot medium was achieved by the supplementation of 1.0 mg/l BAP+0.2mg/l IAA medium (42.22%).

Toucan genotype showed a very good shoot formation and recorded the highest regeneration ability (43.33%), (Fig. 2), followed by FD 0719 genotype (Fig. 3) and Univers genotype (Fig. 4). Data also showed that the interaction between studied media and genotypes no significantly effect. Generally, it is noted that, regenerated shoot percentage varied depending on the sugar beet genotypes and the combination of growth regulators added to the regeneration medium (Pazuki, *et al*. 2018).

Table 5. Effect of different media on regenerated shoot percentage from calli of three sugar beet genotypes

Media mg/l	Genotypes			Mean
	FD 0719	Toucan	Univers	
0.5 mg/l BAP + 0.1 mg/l IAA	30.00^{a}	36.67^{a}	29.67^{a}	**31.11^{B}**
1.0 mg/l BAP + 0.2 mg/l IAA	40.00^{a}	53.33^{a}	33.33^{a}	**42.22^{A}**
1.5 mg/l BAP + 0. 3 mg/l IAA	26.67^{a}	40.00^{a}	30.00^{a}	**32.22^{B}**
Mean	**32.22^{B}**	**43.33^{A}**	**30.00^{B}**	

Means with the same letter within each row or column are not significantly different at p=0.05 according to Duncan's Multiple Range Test.

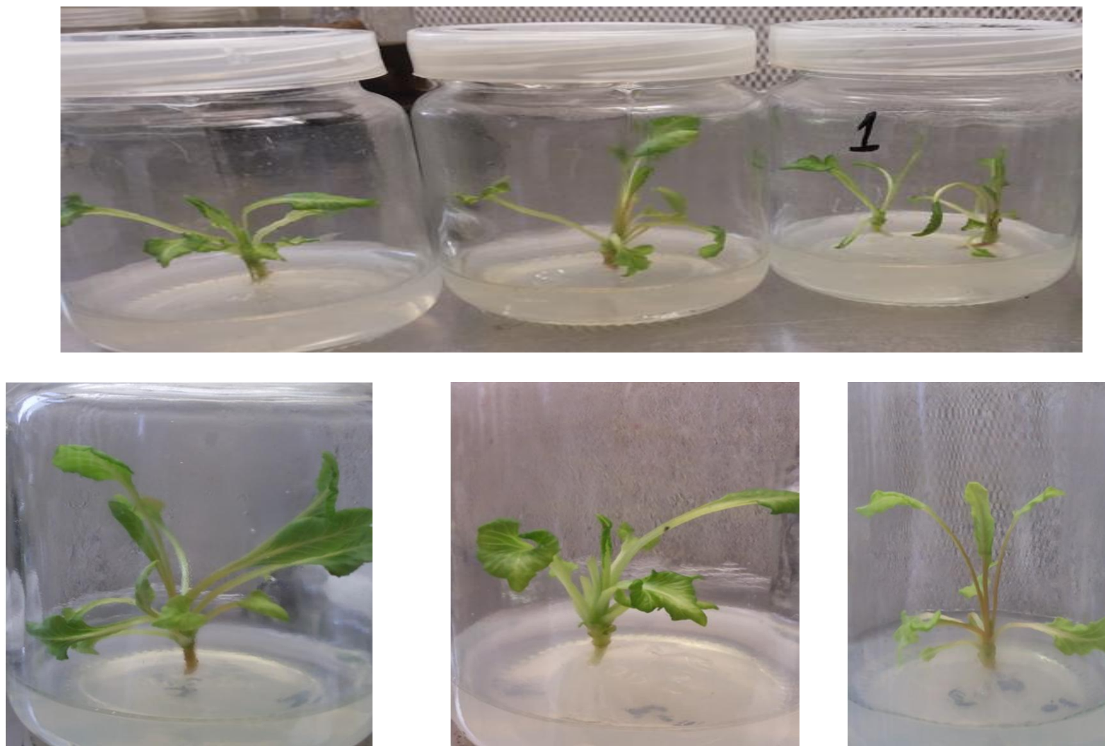


Fig. 3. Shoots developed on half-strength MS-medium containing1.0 mg/l BAP+0.2 mg/l IAA after eight weeks for FD 0719 genotype.

Shoots that regenerated were transformed to anther rooting media. Data in Table (6) showed that MS-medium supplemented with 3.0 mg/l IBA + 0.3mg/l NAA was better than the other media in rooting percentage (80.95%). On the other hand, there were no significantly differences among the three studied genotypes for root formation percentages, as well as, the interaction between media and genotypes. Numerous examples (Hu and Wang, 1983; Konwar and Coutts, 1990; Jacq *et al*. 1992; Gurel, 1997; Mezei *et al*. 2006 and Abdrabou *et al*. 2017) reveal that formation of adventitious shoots in vitro depends on a high cytokinin to a low auxin while formation of adventitious roots depends on a low cytokinin to a high auxin ratio. In our case, IBA and NAA promoted root development. This can be attributed to the possibility of mutations in the cultivation of callus, but can be avoided by the apical meristem culture (Hu, and Wang, 1983 and Miedema, 1982).

Table 6: Root formationpercentage of three sugar beet genotypes cultured on three different MS-medium

Media mg/l	**Genotypes**			**Mean**
	FD 0719	**Toucan**	**Univers**	
1.0 mg/l IBA + 0.1 mg/l NAA	28.57^{a}	42.86^{a}	57.14^{a}	42.86^{B}
2.0 mg/l IBA + 0.2 mg/l NAA	57.14^{a}	85.71^{a}	57.14^{a}	66.66^{AB}
3.0 mg/l IBA + 0.3 mg/l NAA	71.43^{a}	85.71^{a}	85.71^{a}	80.95^{A}
Mean	52.38^{A}	71.43^{A}	66.66^{A}	

Means with the same letter within each row or column are not significantly different at p=0.05 according to Duncan's Multiple Range Test.

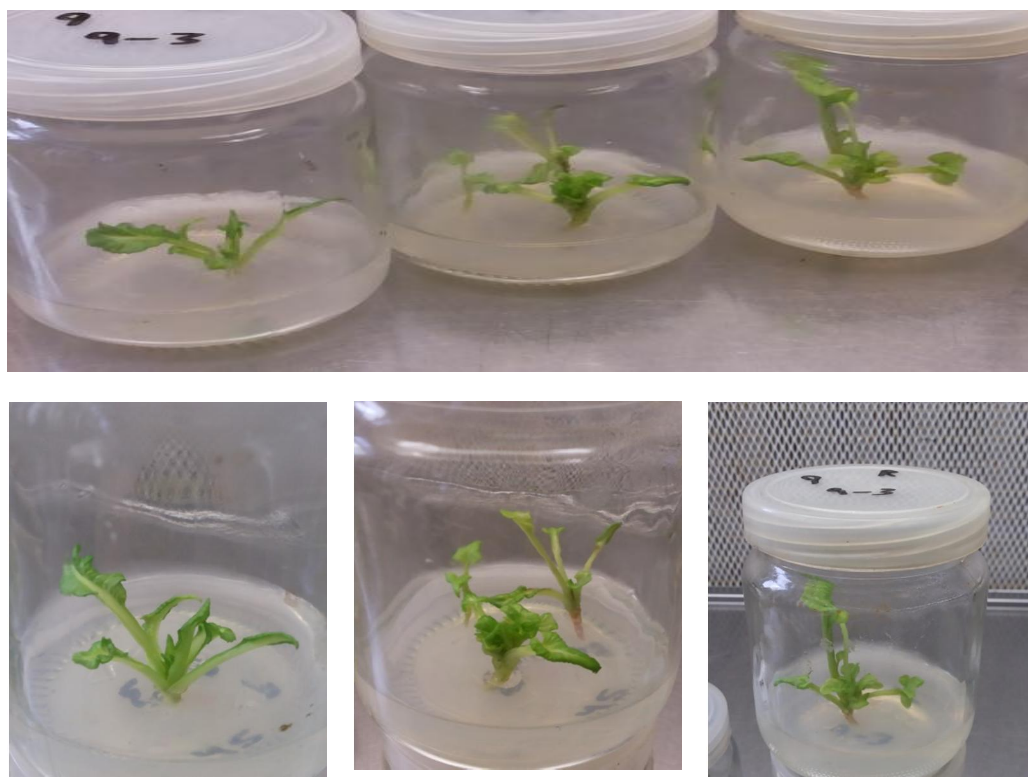


Fig. 4: Shoots developed on half-strength MS-medium containing1.0 mg/l BAP + 0.2 mg/l IAA after eight weeks for Univers genotype.

2. Morphological traits of sugar beet under acclimation

Plants from true seeds (Table 7) showed that FD 0719 genotype had the significant superiority over the other genotypes in root length, diameter and root fresh weight/plant. However, Universes genotype surpassed the other genotypes in total soluble solids percentage (TSS %). Plants from tissue culture technique showed that the evaluated genotypes of sugar beet significantly differed in the studied traits. Toucan genotype surpassed significantly over the other genotypes in root length and (TSS %), however the biggest diameter and highest root weight was recorded by FD 0719 genotype. The results indicated that little differences between sugar beet plants from true seeds and plants from tissue culture. These findings are in line with those reported by (Abd El-Aal, *et al*. 2010; Al-Labbody, *et al*. 2012 and Afez 2016).

Table 7: Comparison between plants of sugar beet from true seeds and from tissue culture plantlet

Genotypes	Root length (cm)	Root Diameter (cm)	Root fresh weight (kg)	Total soluble solids percentage (TSS %)
	Plants of sugar beet from true seeds			
Universes	31.1	11.72	0.878	20.41
Toucan	29.2	12.75	0.784	19.87
FD 0719	34.2	13.86	1.214	18.12
LSD at 0.05	0.41	0.32	0.08	0.21
	Plants of sugar beet from tissue culture technique			
Universes	27.11	10.8	0.710	18.10
Toucan	30.60	9.28	0.621	19.17
FD 0719	28.22	11.4	0.770	19.03
LSD at 0.05	0.65	0.24	0.10	0.15

3. Molecular characterization of the ten sugar beet genotypes

Based on RAPD analysis by using five primers out of twenty RAPD primers produced scorable banding patterns for the ten sugar beet genotypes, the banding patterns exhibited by primer R-2 against the ten genotypes are shown in Figure (5.a) and their densitometric analysis is presented in Table (8). Eleven bands were generated ranging in molecular size (MS) between 94 and 940 bp. All bands were polymorphic with a polymorphism of 100%. For primer R-1the banding patterns exhibited against ten genotypes are shown in Figure (5.b) and their densitometric analysis is presented in Table (8). Thirteen bands were generated ranging in molecular size (MS) between 90 and 950 bp. All bands were polymorphic with a polymorphism of 92.4%. For primer O-2 the banding patterns exhibited against the ten genotypes are shown in Figure (5.C) and their densitometric analysis is presented in Table (9). Thirteen bands were generated ranging in molecular size (MS) between 110 and 990 bp. All bands were polymorphic with a polymorphism of 88.9%. Moreover, the banding patterns exhibited by primer C-20 against the ten genotypes are shown in Figure (5.d) and their densitometric analysis is presented in Table (10). Thirteen bands were generated ranging in molecular size (MS) between 340 and 1050 bp. All bands were polymorphic with a polymorphism of 100%. In addition, for Primer AN1 the banding patterns exhibited against the ten genotypes are shown in Figure (5.e) and their densitometric analysis is presented in Table (11). Thirteen bands were generated ranging in molecular size (MS) between 380 and 1000 bp. All bands were polymorphic with a polymorphism of 100%.

Table 8: DNA polymorphism of the ten Sugar beet genotypes and with primer R-2

No	MS	Genotypes									
	bp	1	2	3	4	5	6	7	8	9	10
1	940	0	1	1	1	1	1	1	1	1	1
2	850	0	0	1	1	1	1	1	1	1	1
3	800	0	1	0	0	0	0	0	0	0	0
4	750	0	1	0	0	0	0	1	0	0	1
5	700	1	0	1	1	1	1	0	1	1	1
6	600	1	0	1	1	1	1	0	1	1	1
7	510	0	0	1	1	1	1	0	1	1	1
8	390	1	0	0	0	0	0	0	0	0	0
9	220	1	0	0	0	0	0	0	0	0	0
10	200	0	1	1	1	1	1	1	1	1	1
11	94	0	1	1	1	1	1	1	1	1	1
Total No. of bands		4	5	7	7	7	7	5	7	7	8

1= FD 0719, 2= Despre 2, 3= Tenor, 4= Bts 301, 5= Bts 237, 6= Franando, 7= Toucan, 8= Sibel, 9= Univers, 10= LLP Ⅲ

Table 9: DNA polymorphism of the ten sugar beet genotypes and with primer R-1

No	MS	Genotypes									
	bp	1	2	3	4	5	6	7	8	9	10
1	950	0	1	1	1	1	1	0	1	1	1
2	920	0	1	1	0	0	1	0	1	0	0
3	850	0	1	1	1	1	1	0	1	1	0
4	800	0	0	0	0	0	1	0	0	0	1
5	715	0	1	1	1	1	1	0	1	1	1
6	600	1	1	1	1	1	1	0	1	1	1
7	450	0	1	1	1	1	1	1	1	1	0
8	400	1	0	0	0	0	0	0	0	0	1
9	380	1	1	1	1	1	1	1	1	1	1
10	230	1	0	0	0	0	0	0	0	0	1
11	160	1	1	1	1	1	1	1	1	1	0
12	140	1	1	1	1	1	1	0	0	1	0
13	90	0	0	0	0	0	0	0	0	0	1
Total No. of bands		6	9	9	9	8	10	3	8	8	8

1= FD 0719, 2= Despre 2, 3= Tenor, 4= Bts 301, 5= Bts 237, 6= Franando, 7= Toucan, 8= Sibel, 9= Univers, 10= LLP Ⅲ

Table 10: DNA polymorphism of the ten Sugar beet genotypes and with primer O-2

No	MS bp	Genotypes									
		1	2	3	4	5	6	7	8	9	10
1	990	0	1	1	1	1	0	1	1	0	1
2	850	1	1	1	1	1	1	1	1	1	1
3	750	1	0	0	0	1	0	1	1	1	0
4	720	0	0	0	0	1	0	0	0	0	0
5	700	0	1	1	1	1	1	1	1	1	1
6	670	0	1	1	1	1	1	1	1	1	1
7	610	1	0	1	1	1	1	1	1	1	1
8	420	0	1	1	1	1	1	1	1	1	1
9	110	0	1	1	1	1	1	0	0	1	1
Total No. of bands		3	6	7	7	9	6	7	7	7	7

1= FD 0719, 2= Despre 2, 3= Tenor, 4= Bts 301, 5= Bts 237, 6= Franando, 7= Toucan, 8= Sibel, 9= Univers, 10= LLP Ⅲ

Table 11: DNA polymorphism of the ten Sugar beet genotypes and with primer C-20

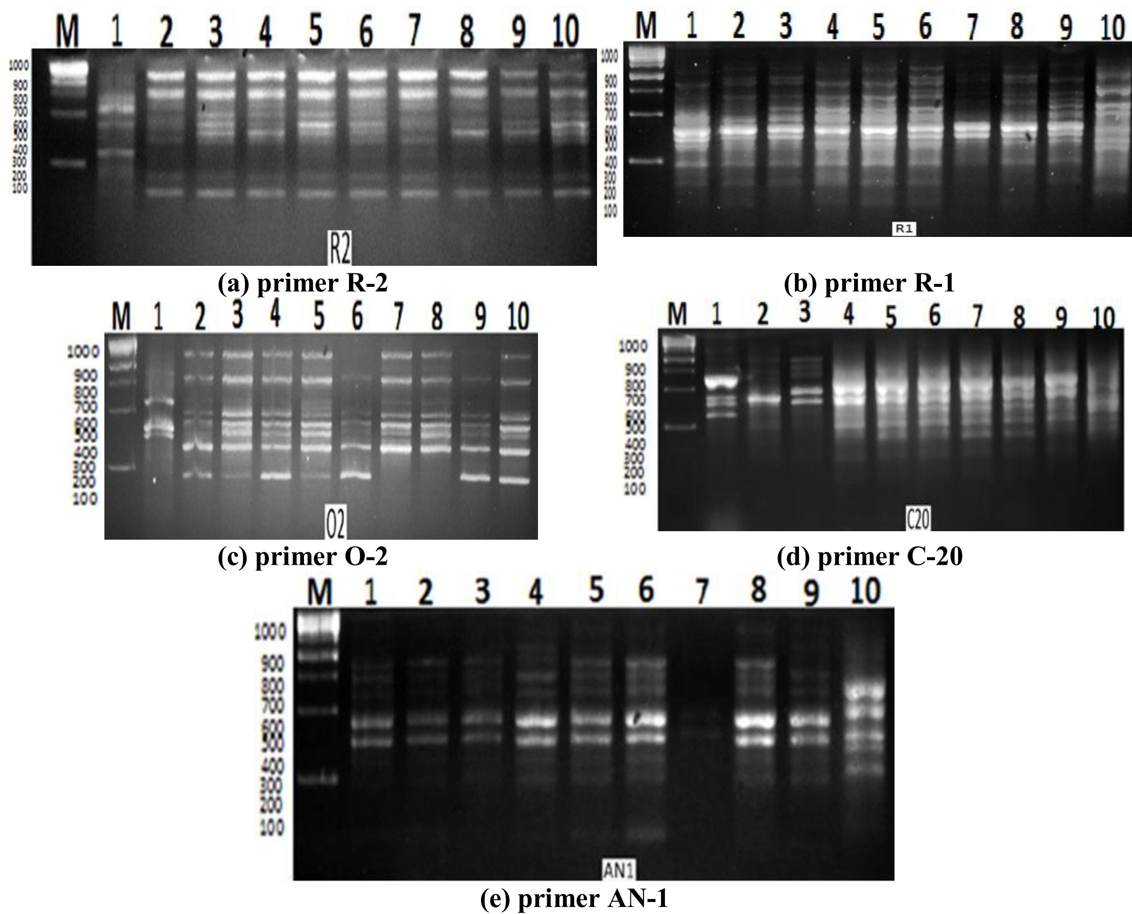
No	MS bp	Genotypes									
		1	2	3	4	5	6	7	8	9	10
1	1050	1	1	0	0	0	0	0	0	0	0
2	900	1	1	1	0	0	0	0	0	0	1
3	890	1	1	1	1	0	0	0	0	0	0
4	850	1	0	0	0	0	0	0	0	0	1
5	820	0	0	1	1	1	1	1	1	1	0
6	790	1	1	1	1	1	1	1	1	1	0
7	660	1	0	0	0	1	1	1	1	0	1
8	600	0	1	0	1	1	1	1	1	1	0
9	500	0	0	0	0	1	1	1	1	0	0
10	340	0	0	0	1	1	1	1	1	1	0
Total No. of bands		6	5	4	5	6	6	6	6	4	3

1= FD 0719, 2= Despre 2, 3= Tenor, 4= Bts 301, 5= Bts 237, 6= Franando, 7= Toucan, 8= Sibel, 9= Univers, 10= LLP Ⅲ

Table 12: DNA polymorphism of the ten Sugar beet genotypes and with primer AN-1

No	MS bp	Genotypes									
		1	2	3	4	5	6	7	8	9	10
1	1000	0	0	0	0	0	0	0	1	0	0
2	990	0	0	0	1	0	0	0	0	1	0
3	960	0	1	1	0	1	1	0	1	0	0
4	850	1	0	0	0	0	0	0	0	0	0
5	800	1	0	0	1	1	1	1	1	1	0
6	770	1	1	0	1	1	1	1	1	1	1
7	700	0	0	0	0	0	0	0	0	0	1
8	660	1	1	1	1	1	1	0	1	1	0
9	640	1	1	1	1	1	1	0	1	1	1
10	615	0	0	0	1	1	1	0	1	1	1
11	500	0	0	0	1	1	1	0	1	1	1
12	380	0	0	0	0	1	1	0	0	0	0
Total No. of bands		7	4	3	7	8	8	2	8	7	5

1= FD 0719, 2= Despre 2, 3= Tenor, 4= Bts 301, 5= Bts 237, 6= Franando, 7= Toucan, 8= Sibel, 9= Univers, 10= LLP Ⅲ



(a) primer R-2 **(b) primer R-1**
(c) primer O-2 **(d) primer C-20**
(e) primer AN-1

Fig. 5: DNA polymorphism of the ten Sugar beet genotypes amplified with RAPD primers
1= FD 0719, 2= Despre 2, 3= Tenor, 4= Bts 301, 5= Bts 237, 6= Franando, 7= Toucan, 8= Sibel, 9= Univers, 10= LLP Ⅲ

Table (13) summarizes the number of generated bands using each RAPD-primer among the 55 amplified fragments across the ten Sugar beet genotypes, with the five RAPD-primers. Almost all bands were polymorphic with polymorphism percentage about 96.3%.
Detection of variants at the DNA level is of immense importance in order to utilize in vitro selected lines in crop improvement. The variants may have genetic or epigenetic basis therefore, early detection of these is of prime importance. It is rather difficult to detect the genetic variation from morphological features Hallden, *et al.* (1994).

Table 13: Total number of bands (Monomorphic and Polymorphic), Polymorphism percentages revealed by five RAPD primers for the ten Sugar beet genotypes

Primer name	Total bands	Monomorphic bands	Polymorphic bands	Polymorphism content
R-2	11	0	11	100%
R-1	13	1	12	92.4%
O-2	09	1	08	88.9%
C-20	10	0	10	100%
AN-1	12	0	12	100%
Total	55	2	53	

The RAPD data were used to estimate the genetic similarity values among the ten Sugar beet genotypes regenerates by using UPGMA computer analysis (Table 13 and Fig. 6).

The highest similarity value between the ten Sugar beet genotypes (0.955) were recorded between genotype 4 and genotype 9, followed by 0.919 and 0.904 that observed between genotype 9 and both genotype 4 and genotype 5 respectively. However, the lowest similarity value was observed between genotype 7 and genotype 1 (0.383).

A dendogram for the genetic relationship among the ten sugar beet genotypes is illustrated in Fig. (6), as they were separated into two major groups, the first one including only genotype 1, and the second one divided to two subgroups, the first one including only genotype 10, and the other one divided to other two subgroups, the first one including only genotype 7, and the other one divided to other two subgroups, the first one including both genotypes 2 and 3, and the second one divided to other two subgroups, the first one including both 4 and 9 genotypes, and the second one divided to other two subgroups, the first one including only genotype 8, and the second one including both genotypes 5 and 6.

The clustering results and the genetic variation within and between the studied genotypes and the degree of gene differentiation suggest a relatively high genetic diversity that can be used in rapeseed breeding programs. Considering the diversity of the genotypes, the genotypes with a wide genetic distance may be used as parents to crosses for taking advantage of heterosis and for making mapping populations in the QTL mapping studies. In this study, the RAPD molecular data provided a lot of information.

Table 14: Similarity value (Pairwise comparison) of the ten sugar beet genotypes based on RAPD data.

Pairwise comparison Matrix

Genotypes	Matrix File Input									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	1.000									
2	.453	1.000								
3	.481	.814	1.000							
4	.483	.730	.844	1.000						
5	.484	.687	.794	.889	1.000					
6	.459	.697	.806	.873	.933	1.000				
7	.383	.577	.566	.667	.721	.667	1.000			
8	.467	.677	.788	.857	.919	.904	.746	1.000		
9	.491	.677	.794	.955	.901	.886	.679	.870	1.000	.
10	.473	.567	.656	.677	.667	.676	.519	.657	.656	1.000

1= FD 0719, 2= Despre 2, 3= Tenor, 4= Bts 301, 5= Bts 237, 6= Franando, 7= Toucan, 8= Sibel, 9= Univers, 10= LLP Ⅲ

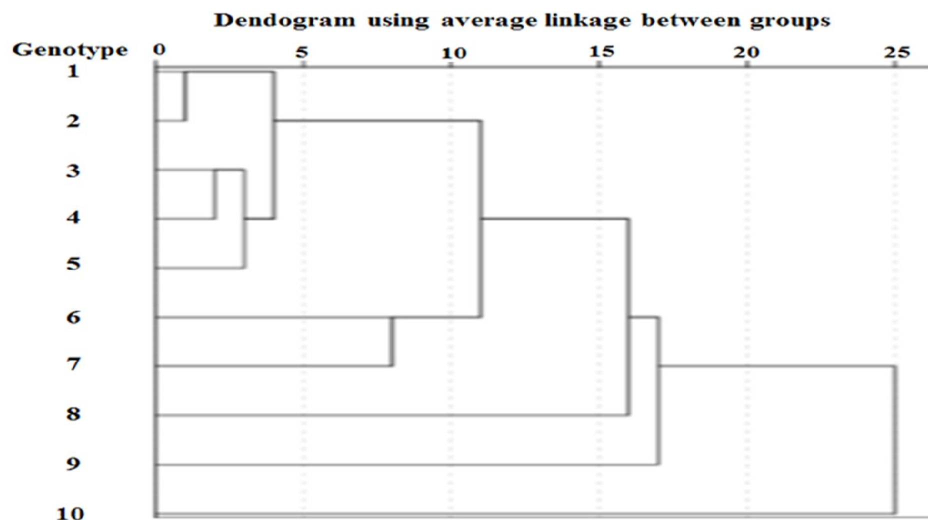


Fig. 6: Dendogram of the phylogenetic relationships among the ten sugar beet genotypes

Conclusion

Our results indicate that cotyledon explants are good starting materials for the *in vitro* production of shoots in sugar beet. White and friable callus was derived from hypocotyl, while, cotyledon explant produced green and compact callus, the first one can be used in cell suspension culture application. Callus was shown to be capable of developing vegetative shoots on a half-strength MS-medium contained 1.0 mg/l BAP+0.2 mg/l IAA and the regenerated shoot capable of rooting on a half-strength MS-medium supplemented with 3.0 mg/l IBA+0.3 mg/l NAA. Genetic variation was investigated for the ten sugar beet genotypes. Data suggest a relatively high genetic diversity among them. The highest similarity value among the ten sugar beet genotypes (0.955) was recorded between Bts 301genotype and Univers genotype, however, the lowest similarity value was observed between Toucan genotype and FD 0719 genotype (0.383).

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