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VARIATION INDUCED THROUGH TISSUE CULTURE IN SUNFLOWER (*Helianthus annuus* L.)

Clara R. Azzam

Cell Research Dept., Field Crops Research Institute, Agricultural Research Center

ABSTRACT

This work aims to evaluate the variations induced through tissue culture technique (somaclonal variation) for the progenies of the regenerated (R_0) plants derived from different explants (immature embryos, hypocotyls, cotyledons and leaves) of two sunflower entries; Miak cv. and a synthetic population (SP) in R_1 and R_2 generations. R_1 and R_2 plants were grown in an experimental field at Giza, and some morphological characters, yield and yield components, oil content and protein fingerprints were evaluated. The mean values of the studied characters in the two generations for the two entries varied according to the explant source. Plants produced from tissue culture through somaclonal variation were significantly superior in almost all of growth characters, yield and yield components and oil content. The differences were significant for plant height, number of leaves /plant, number of seeds/plant, weight of seeds/ head, seed index and oil % in both generations and entries. Oil percentage increased by 39.3%, 37.6%, 36.4% and 32.0%, as compared with the control, for progenies derived from immature embryo of Miak and the SP in R_1 and R_2, respectively. The coefficient of variation varied according to the characters studied and genotype in R_1 and R_2 generations. The highest phenotypic coefficient of variability values were detected for number of seeds and weight of seeds/head in both R_1 and R_2. The obtained variations may support the evidence of effectiveness of selection to improve some sunflower characters in subsequent generations. Seed storage proteins banding patterns (SDS-PAGE) was successful in generating biochemical genetic markers related to variations induced through tissue culture. Protein fingerprints of the progenies of in vitro regenerated sunflower plants revealed great variations in configurations and numbers of bands appear as appearance and disappearance of new protein bands. The results indicate that induced somaclonal variation through tissue culture techniques is useful to induce variation in sunflower.

Key words: ***Sunflower, Helianthus annuus, Tissue culture, Somaclonal variation, Biochemical genetic markers, Protein electrophoresis, SDS-PAGE.***

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is an important source of edible vegetable oil in the world because it has high unsaturated fatty acids and vitamin E contents (Gosal *et al* 1988). The major breeding objectives of sunflower are directed towards increasing seed productivity, oil content and

quality as well as resistance to birds, insects and diseases to decrease the gap between the consumption and importation of edible vegetable oil.

Variation induced through different breeding methodologies is of great interest to the plant breeder. The success of any crop improvement program depends on the extent of genetic variability in the base population (Bajaj 1990). The assembly of genetic variability is vital to any plant breeding enterprise. Without a continual input of "new" genes, progress, as measured by improved agronomic suitability, cannot be made (Larkin and Scowcroft, 1981). The frequency of genetic changes is significantly higher for somaclonal variation than observed for spontaneous genetic changes. The plant regeneration step acts as a sieve that eliminates most deleterious genetic changes. Moreover, DNA rearrangement and different isoenzymatic patterns could be generated by somaclonal variation (McCoy and Bingham 1977, Baertlein and McDaniel 1987, Bajaj 1990 and Rattray 1991).). These features make somaclonal variation on attractive approach for crop improvement in modern plant breeding programs. The challenge and opportunity is to create valuable new raw materials that decrease the gab between the production and the consumption.

Thus, successful applications of new methods of biotechnology can induce new types of variability (such as somaclonal variation) for selection of new genotypes. Consequently, it contributes to overcoming the problem of narrow genetic base of cultivated species. Such new approaches could accelerate the breeding process, especially in oil crops (Greco *et al* 1984, Jones 1988 and Friedt 1996).

Somaclonal variation can also be visualized as a form of mutation breeding which might offer hope for plant improvement (Larkin and Scowcroft 1981, Evans 1989 and Larkin *et al* 1989). When plants are regenerated from somatic cells via cell culture, many plants show genetic variability. Unfortunately, some of these variability may be epigenetic and transient, and therefore not useful for crop improvement, as it is not transmitted through meiosis. However, stable genetic changes are common and if useful, can be of interest to plant breeders.

Media requirement for regeneration in sunflower varied with explant source and for both Miak and SP genotypes, hypocotyl explant was the best source for obtaining high regeneration response as compared to the other tested explants; immature embryos, cotyledons and leaves (Azzam 2000 and Azzam *et al* 2003). Moreover, hypocotyl explant is the best in obtaining the highest regeneration ability of calli. SP was the best genotype in regeneration

ability from immature embryos, cotyledons and young hypocotyls. (Azzam 2000 and Amer *et al* 2003). Santos and Caldeira 1998 also found that hypocotyl was the best explant for regeneration in sunflower.

Protein markers were among the first group of molecular markers exploited for genetic diversity assessment. Seed storage protein (water-soluble fraction).

Thus, the aim of the present study was to evaluate the variations induced through tissue culture techniques (somaclonal variation) in the progenies of the regenerated (R_0) plants, from different explants (immature embryos, hypocotyls, cotyledons and leaves), of sunflower entries; Miak cv. and SP in R_1 and R_2 generations, with regard to some morphological characters, yield and yield components, oil content and protein fingerprints.

MATERIALS AND METHODS

The seeds used in this study were obtained from regenerated plants (R_0), which produced after culturing four explants of two sunflower entries; Miak cv. and SP through tissue culture as developed previously by Azzam *et al* (2003). The genotype synthetic population (SP) provided by Agronomy Dept., Faculty of Agriculture, Cairo Univ. (Shabana 1990), while Miak cv. (an open pollinated cultivar) obtained from Oil Crops Research Dept., ARC.

The seeds were grown to arise R_1 generation then R_2 regeneration in two field experiments carried out at the Agricultural Research Center, Giza (loamy clay soil) during two successive seasons (2001 and 2002). Nine populations were grown each generation (no regeneration was observed in SP using cotyledon explant on all hormones combination and concentrations). The experimental design was randomized complete block design (RCBD) with four replicates. Every plot consisted of 10 rows, 3.0 m long. Seeds were planted in hills, 20 cm apart within rows, and 70 cm between rows. Plants were thinned 21 days after emergence and two plants were left per hill. All other agriculture practices were applied as recommended. Fifteen plants were taken randomly from the inner rows and the following data were recorded on an individual plant basis: plant height (cm), no. of leaves /plant, leaf area (cm^2), head diameter (cm), no. of fertile seeds/head, weight of fertile seeds/head (g.) and oil content % (using NMR according to Marquard 1987).

Statistical analysis for RCBD was separately carried out for each genotype and generation according to the models described by Steel and Torrie (1980). Least significant differences (L.S.D.) test was computed to

compare differences among means of populations derived from different explants at 5% level. Variability in R_1 and R_2 generations was assessed as range (Min. and Max.), phenotypic (P.C.V. %) and genotypic (G.C.V. %) coefficients of variability, heritability in the broad sense (h^2 %) and genetic advance under 10 % selection intensity (Gs 10%), according to Allard (1999).

Seed storage protein electrophoresis "sodium dodecylesulfate polyacrylamide gel electrophoresis" (SDS-PAGE) was used to assess polymorphisms in the progenies of two sunflower entries as affected by somaclonal variation induced through tissue culture of different explants: immature embryo, hypocotyl, cotyledon and leaf in R_1 and R_2. Protein fingerprinting was achieved using water-soluble protein fraction. Protein fractionation was performed on a vertical slab gel, using the electrophoresis apparatus "Hoefer E600" manufactured by Amersham-pharmacia biotech.

Protein extraction was performed according to Laemmli (1970). Gels were stained with commassie brilliant blue R-250 solution, photographed and scored using gel documentation system manufactured by Alpha Ease FC (Alphimager 2200), U.S.A.

In (Ro) plants, some variations in morphological characters were detected such as plant height and root length at seedling stage in the laboratory and plant height, root length, head diameter and flower's color at maturity stage in greenhouse as reported by Azzam *et al* (2003) and shown in Fig. (1).

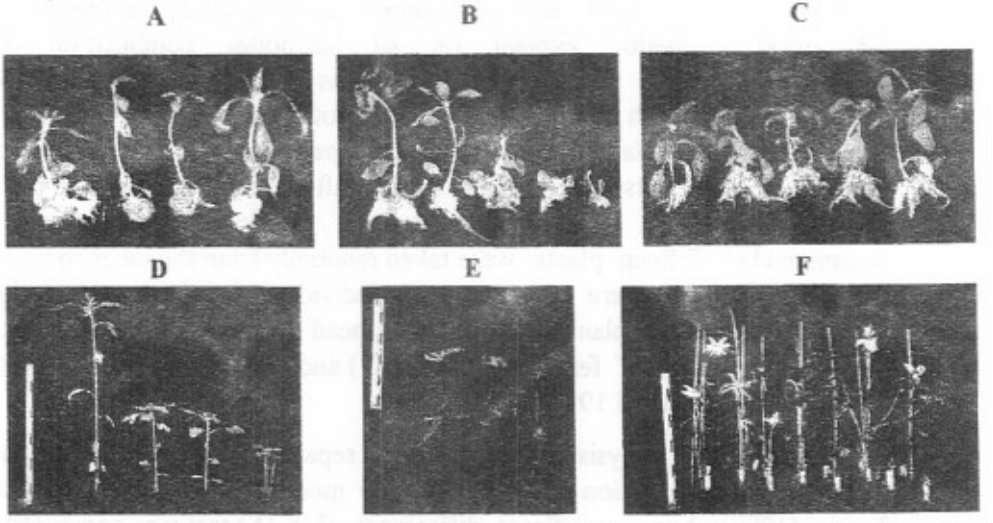


Fig .1. Variations induced in R_0 plants at the seedling growth stage in laboratory (A, B and C) and at the maturity stage in green house (D, E and F).

RESULTS AND DISCUSSION

Changes in mean values of the progenies derived from different explants for the two sunflower entries in R_1 and R_2 generations are illustrated in Tables (1) and (2).

Plant height decreased significantly in both R_1 and R_2 generations for both entries in all plants derived from immature embryos, hypocotyls, cotyledons and leaves except the regenerated populations derived from Miak leaf and cotyledon in R_1 generation as shown in Table (1). The opposite was true for number per plant, number of seeds per head, weight of seeds per head, seed index and oil percentage, which significantly increased towards the positive direction in both R_1 and R_2 generations in both entries, except number and weight of seeds per head of the population derived from cotyledon explant of Miak cultivar in both R_1 and R_2 generations, as shown in Tables (1) and (2).

Highly variable organogenic regeneration has been obtained in sunflower, depending upon culture conditions (all growth conditions such as media composition, light, temperature), genotype, explant and their interactions. The interaction between explant, media and genotype resulted in significant increases in most yield and yield component characters in the progenies derived from different explants of both genotypes. Zorzoli *et al* (1998) also observed the same trend.

Head diameter for progenies derived from immature embryo of the SP increased in R_1 and R_2 generations, the increases were 71.5 % and 63.19%, as compared with the control, respectively. While, concerning to the progenies derived from hypocotyl, the increases were 50.1% and 46.4% as compared with the control, respectively. On the other hand, the increases in number of seeds/head were 46.0%, 55.5%, 35.1%, 67.4%, 35.9% and 48.1%, as compared with the control, for progenies derived from hypocotyls of Miak and the SP in R_1, progenies derived from hypocotyls of Miak and the SP in R_2, progenies derived from immature embryo of the SP in R_1 and R_2, respectively. Consequently weight of seeds/head increased significantly, the increases were higher than the control by 63.3%, 29.5%, 52.1%, 32.7%, 42.7%, 68.6 and 34.0% for progenies derived from hypocotyls of Miak and the SP in R_1, progenies derived from hypocotyl of Miak and the SP in R_2, progenies derived from immature embryo of Miak and the SP in R_1 and progenies derived from immature embryo of Miak in R_2, respectively. Moreover, the oil percentage increased significantly, the increases were 39.3%, 37.6%, 36.4% and 32.0%, as compared with the control, for

Table 1. Means of some morphological and yield characters for progenies derived from different explants of two sunflower entries Miak and SP in R_1 generation.

characters	Entries	Explant					Mean	LSD 5%
		Control	Immature embryos	Hypocotyls	Leaves	Cotyledons		
Plant height (cm)	Miak	211.36	164.10	195.00	240.75	216.36	205.51	44.47
	SP	261.88	202.00	183.00	182.88	-	207.44	27.35
	Mean	236.62	183.05	189.00	211.82	216.36		
No. of leaves / plant	Miak	22.95	30.38	31.75	37.25	32.00	30.87	9.41
	SP	34.63	47.63	44.63	44.50	-	42.85	7.37
	Mean	28.79	39.01	38.19	40.88	32.00		
Leaf area (cm^2)	Miak	19.58	33.57	37.90	26.66	34.75	30.49	7.58
	SP	21.95	32.48	37.23	26.65	-	29.58	N.S.
	Mean	20.77	33.03	37.57	26.66	34.75		
Head diameter (cm)	Miak	14.12	15.94	19.50	17.50	17.09	16.83	N.S.
	SP	13.88	23.80	20.83	19.13	-	19.41	6.25
	Mean	14.00	19.87	20.17	18.32	17.09		
No. of seeds/head	Miak	508.25	526.88	741.38	626.00	349.04	550.31	252.61
	SP	623.63	847.50	969.88	744.88	-	796.48	141.89
	Mean	565.94	687.19	855.63	685.44	349.04		
Weight of seeds /head (g.)	Miak	30.87	44.05	50.42	33.74	27.70	37.36	13.49
	SP	40.85	68.87	52.90	51.17	-	53.48	10.61
	Mean	35.86	56.46	51.66	42.455	27.70		
Seed index (g.)	Miak	6.07	8.24	6.30	5.42	6.36	6.48	0.28
	SP	7.91	7.47	5.29	6.56	-	6.81	0.19
	Mean	6.99	7.855	5.795	5.99	6.36		
Oil %	Miak	36.10	50.28	39.26	46.04	37.05	41.75	0.36
	SP	39.56	54.44	49.17	44.70	-	46.97	0.41
	Mean	37.83	52.36	44.215	45.37	37.05		

ble 2. Means of some morphological and yield characters for progenies derived from different explants of two sunflower entries Miak and SP in R_2 generation.

haracters	Entries	Explant					Mean	LSD 5%
		Control	Immature embryos	Hypocotyls	Leaves	Cotyledons		
nt height n)	Miak	236.00	126.63	208.25	215.25	214.88	200.20	66.44
	SP	248.00	196.38	203.63	182.88	-	207.72	33.82
	Mean	242.00	161.51	205.94	199.07	214.88		
. of leaves / nt	Miak	24.42	31.94	36.83	36.50	33.86	32.71	7.84
	SP	34.88	47.25	46.13	44.38	-	43.16	4.58
	Mean	29.65	39.60	41.48	40.44	33.86		
af area n2)	Miak	24.69	33.32	31.41	30.78	33.39	30.72	N.S.
	SP	22.16	30.86	38.05	28.23	-	29.83	8.37
	Mean	23.43	32.09	34.73	29.51	33.39		
ad diameter n)	Miak	14.64	18.19	18.38	16.88	16.88	16.99	N.S.
	SP	15.16	24.74	22.19	20.05	-	20.54	5.43
	Mean	14.90	21.47	20.29	18.47	16.88		
. of eds/head	Miak	501.17	513.75	677.00	586.00	369.00	529.38	183.09
	SP	596.88	883.75	999.38	737.63	-	804.41	143.92
	Mean	549.03	698.75	838.19	661.82	369.00		
eight of seeds ead (g.)	Miak	32.47	43.51	49.40	35.36	28.87	37.92	13.37
	SP	33.35	36.54	44.26	31.67	-	36.46	8.23
	Mean	32.91	40.03	46.83	33.52	28.87		
ed index (g.)	Miak	6.28	8.38	7.13	6.25	6.50	6.91	0.32
	SP	5.61	3.92	4.13	4.12	-	4.45	0.19
	Mean	5.95	6.15	5.63	5.19	6.50		
il %	Miak	37.58	51.27	41.01	36.51	45.72	42.42	0.43
	SP	40.17	53.04	42.27	44.43	-	44.98	0.33
	Mean	38.88	52.16	41.64	40.47	45.72		

progenies derived from immature embryo of Miak and the SP in R_1, progenies derived from immature embryo of Miak and the SP in R_2, respectively. Similar results were obtained by Coughlan *et al* (1998) and Zorzoli *et al* (1998). These results indicate that wide variation in oil % was existed allowing selection of this trait and these genotypes with the high ability to oil production, which developed through somaclonal variation, are considered promising and providing an array of new source of oils.

Some of the morphological changes were identified in R_1 with respect to number of heads such as dihead and polyflower plants. However, those changes did not appear in R_2 as shown in Fig (2). Consequently, these changes may be due to physiological changes. Moreover, variation in flower's color (varied between light yellow, dark yellow, yellow with orange shadow and orange) and seed's size and color were also identified as shown in Fig (2).

The genetic parameters estimated for cv. Miak and the SP are presented in Tables (3 and 4). Wide ranges and high values of phenotypic coefficient of variability were detected in populations derived from different sunflower explants through somaclonal variation especially in the SP. The range of leaf area, No. of seeds/plant and weight of seeds/plant for Miak cultivar and the SP was extended towards a positive direction for all explants in both R_1 and R_2 generations. Also, a wide range of all studied characters for the SP was found in populations derived from immature embryo and hypocotyl explants in both generations. High coefficient of variation for leaf area was noticed at the populations derived from immature embryos in the SP in both R_1 and R_2, and in R_2 for the weight of seeds/ head. These findings indicate that effective selection for this trait could be achieved in the SP in further generations.

The Data also show high Gs and h^2 in almost all studied characters in R_2 for both entries. The results may support the evidence of effectiveness of selection to improve some sunflower characters in subsequent generations.

Electrophoretic separation of water soluble extracted protein in the studied nine populations derived from the two sunflower entries in R_1 and R_2 generations are shown in Fig. (3) and Fig. (4) and their densitometric analyses are illustrated in Tables (5) and (6), respectively. A positive sign in the Tables represents the presence of each corresponding band. By contrast, a negative sign represents the absence of each corresponding band.

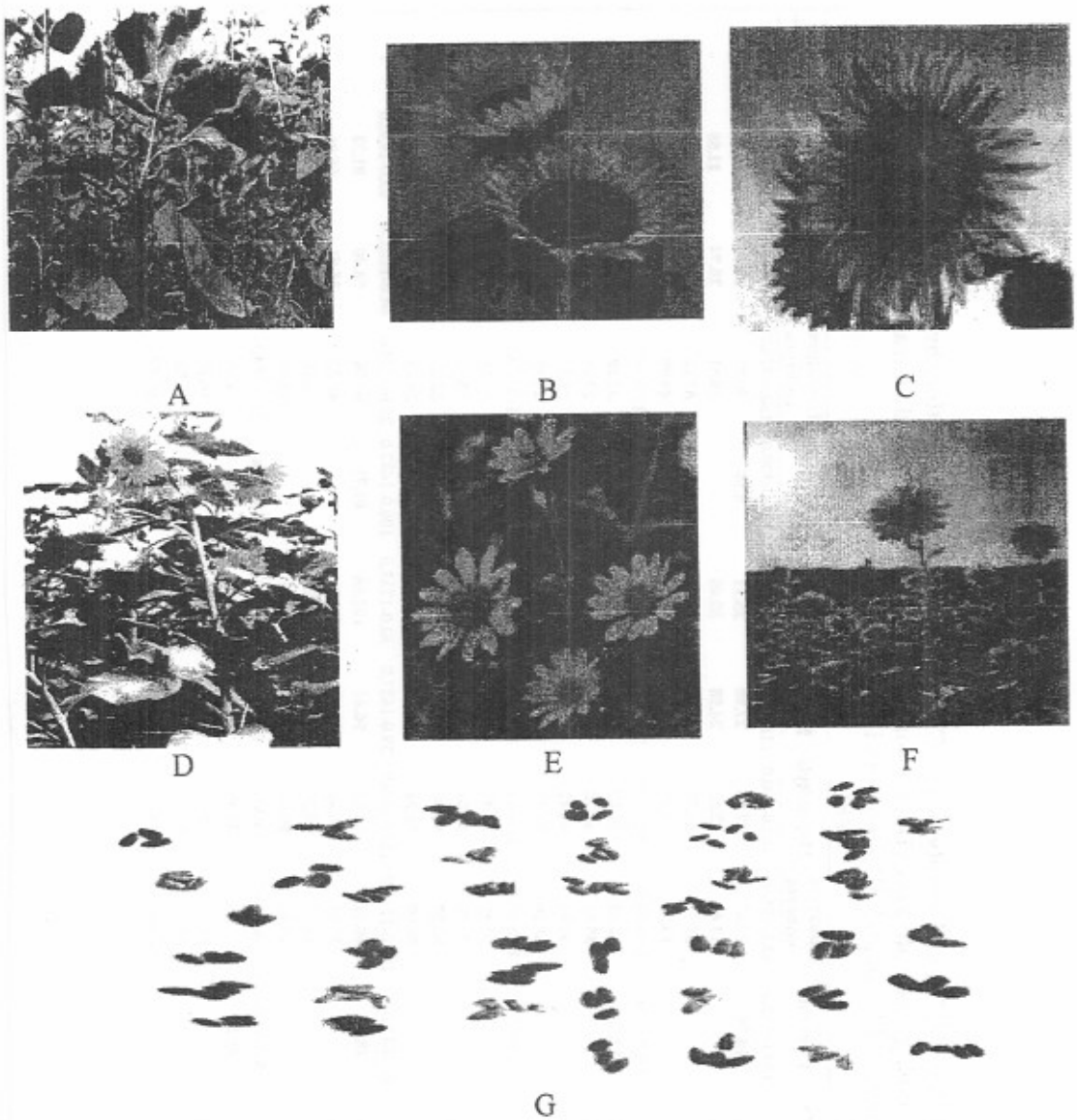


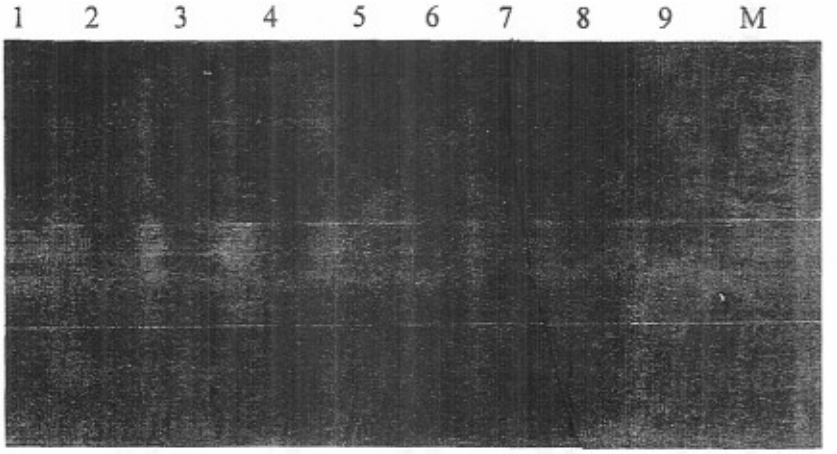
Fig. 2. **Some of the morphological changes identified in the R_1 generation, grown in the field, (A): plants without heads – (B and C): diheaded plants – (D and E) polyflower plants. And (F): variations of plant height– (G) variations in seeds size and color.**

Table 3. Phenotypic (P. C. V. %) and genotypic (G. C. V. %) coefficient of variation, broad sense heritability (h^2 %) and expected genetic advance (Gs) for some morphological and yield characters for progenies of different explant of Miak and SP genotypes in R_1 generation.

Characters	Genetic parameters	Miak					SP				
		Control	Immature embryos	Hypocotyls	Leaves	Cotyledons	Control	Immature embryos	Hypocotyls	Leaves	Cotyledon
Plant height (cm)	Range	155.0-255.0	88.0-195.0	105.0-290.0	111.0-309.0	105.0-320.0	229.0-300.0	150.0-326.0	133.0-305.0	141.0-282.0	-
	P. C. V.%	14.79	23.67	27.53	27.98	25.11	8.68	30.87	31.21	25.71	-
	G. C. V.%		13.41	22.52	24.58	20.48		28.81	28.74	22.58	-
	h^2%		32.11	66.95	77.15	66.50		87.10	84.80	77.11	-
	Gs		13.30	32.25	37.78	29.23		47.05	46.32	34.69	-
No. of leaves / plant	Range	12.0-22.0	19.0-52.0	20.0-49.0	25.0-53.0	20.0-45.0	20.0-45.0	27.0-70.0	17.0-66.0	15.0-75.0	-
	P. C. V.%	12.19	40.31	31.68	22.32	20.94	23.49	32.39	37.48	44.52	-
	G. C. V.%		39.61	30.78	21.40	19.57		27.69	32.73	40.71	-
	h^2%		96.53	94.42	91.93	87.36		73.07	76.26	83.62	-
	Gs		68.10	52.35	35.90	32.01		41.42	50.03	65.15	-
Head diameter (cm)	Range	11.0-17.0	10.5-25.0	11.0-31.0	10.0-29.0	9.5-30.0	9.0-22.0	11.0-34.0	7.0-27.5	9.0-30.0	-
	P. C. V.%	13.27	33.20	32.40	36.74	26.47	32.85	32.25	36.64	40.09	-
	G. C. V.%		30.82	30.89	35.12	24.11		25.44	28.26	31.89	-
	h^2%		86.22	90.90	91.35	82.98		62.24	59.48	63.28	-
	Gs		50.09	51.54	58.73	38.44		35.12	38.14	44.40	-
No. of seeds/ plant	Range	60.0-1136.0	100.0-1115.0	133.0-806.0	39.0-1687.0	30.0-1238.0	100.0-1487.0	28.0-1792.0	64.0-1803.-0	63.0-1830.0	-
	P. C. V.%	48.74	60.42	66.63	96.41	102.68	69.71	69.56	58.70	81.32	-
	G. C. V.%		39.61	58.24	87.37	74.43		46.23	35.91	58.06	-
	h^2%		42.99	76.39	82.12	52.54		44.16	37.41	50.98	-
	Gs		45.46	89.08	138.55	94.42		53.76	38.43	72.54	-
Weight of seeds /head (g.)	Range	5.5-62.0	5.0-89.8	9.8-91.1	3.4-70.9	2.6-68.1	5.2-57.4	11.0-184.1	8.4-143.4	4.2-146.0	-
	P. C. V.%	52.29	61.05	46.40	84.47	81.22	45.53	94.55	95.55	97.09	-
	G. C. V.%		49.23	34.22	68.79	57.31		90.48	89.14	90.64	-
	h^2%		65.04	54.38	66.32	49.79		91.58	87.02	87.16	-
	Gs		69.48	44.16	98.03	70.76		151.52	145.52	148.08	-

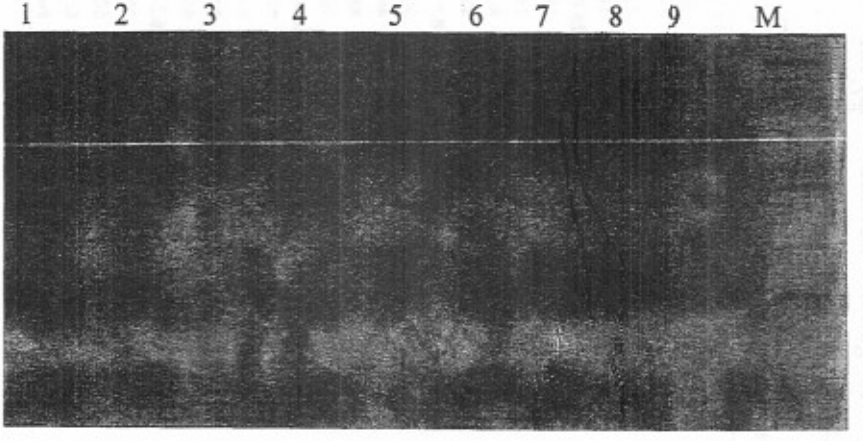
Table 4. Phenotypic (P. C. V. %) and genotypic (G. C. V. %) coefficient of variation, broad sense heritability (h^2 %) and expected genetic advance (Gs) for some morphological and yield characters for progenies of different explant of Miak and SP genotypes in R_2 generation.

Characters	Genetic parameters	Miak					SP				
		Control	Immature embryos	Hypocotyls	Leaves	Cotyledons	Control	Immature embryos	Hypocotyls	Leaves	Cotyledon
Plant height (cm)	Range	175.0-263.0	78.0-205.0	100.0-278.0	115.0-300.0	99.0-290.0	229.0-286.0	133.0-298.0	126.0-275.0	122.0-301.0	-
	P. C. V.%	13.47	36.77	28.91	30.66	30.33	8.46	29.12	29.99	31.67	-
	G. C. V.%		27.71	24.12	26.99	26.44		27.08	28.07	29.56	-
	h^2%		56.79	69.63	77.48	76.01		86.51	87.63	87.15	-
	Gs		36.54	35.23	41.58	40.35		44.09	45.99	48.30	-
No. of leaves / plant	Range	17.0-30.0	21.0-49.0	25.0-53.0	25.0-51.0	25.0-60.0	19.0-48.0	31.0-77.0	22.0-73.0	21.0-68.0	-
	P. C. V.%	15.55	35.50	31.19	27.87	25.80	26.65	33.18	36.62	35.97	-
	G. C. V.%		33.38	29.48	25.91	23.15		27.53	30.75	29.37	-
	h^2%		88.40	89.37	86.45	80.52		68.85	70.54	66.66	-
	Gs		54.92	48.77	42.16	36.35		39.97	45.20	41.96	-
Head diameter (cm)	Range	9.5-20.3	10.0-33.0	10.5-35.0	8.5-36.0	11.9-30.0	10.0-25.0	9.5-43.0	9.9-40.6	11.4-35.0	-
	P. C. V.%	19.42	19.42	39.07	38.40	49.48	32.59	43.24	43.82	41.89	-
	G. C. V.%		35.86	35.09	46.48	23.81		38.17	38.06	33.91	-
	h^2%		84.26	83.47	88.25	66.73		77.95	75.43	65.56	-
	Gs		57.61	56.09	76.42	34.04		58.98	57.85	48.06	-
No. of seeds/ plant	Range	89.0-1022.0	120.0-1000.0	111.0-1265.0	110.0-1276.0	100.0-1080.0	144.0-1229.0	154.0-1956.0	121.0-1982.0	90.0-1745.0	-
	P. C. V.%	43.88	48.03	51.31	74.96	76.57	61.17	67.35	59.73	78.18	-
	G. C. V.%		20.18	39.49	63.85	48.13		53.67	46.90	61.49	-
	h^2%		17.66	59.24	72.55	39.51		63.49	61.64	61.85	-
	Gs		14.84	53.19	95.18	52.94		74.84	64.44	84.62	-
Weight of seeds /head (g.)	Range	11.0-65.8	9.3-81.2	10.2-91.1	6.2-68.1	3.5-77.2	11.0-65.8	7.1-81.2	3.5-91.1	6.2-68.1	-
	P. C. V.%	46.34	52.92	41.10	75.07	78.39	46.63	64.65	59.61	65.92	-
	G. C. V.%		40.29	27.76	60.75	58.54		47.21	48.17	42.83	-
	h^2%		57.96	45.64	65.49	55.77		53.34	65.29	42.21	-
	Gs		53.68	32.82	86.04	76.51		60.34	68.11	48.70	-



1: Miak control
2: Miak (immature embryos)
3 Miak (hypocotyls)
4: Miak (leaves)
5: Miak (cotyledons)
6: SP control
7: SP (immature embryos)
8: SP (hypocotyls)
9: SP (leaves)

Fig. 3. Protein banding patterns of cv. Miak and SP in R_1 generation derived through tissue culture of different sunflower explants.



1: Miak control
2: Miak (immature embryos)
3 Miak (hypocotyls)
4: Miak (leaves)
5: Miak (cotyledons)
6: SP control
7: SP (immature embryos)
8: SP (hypocotyls)
9: SP (leaves)

Fig. 4. Protein banding patterns of cv. Miak and SP in R_2 generation derived through tissue culture of different sunflower explants.

Table 5. Banding patterns of SDS-PAGE for water soluble protein fraction in R_1 generation of populations derived from the two sunflower entries through tissue culture of different explants.

Band number	Molecular weight (KD)	Fig. (4)								
		Miak cv.					SP			
		Control	Immature embryos	Hypocotyls	Leaves	Cotyledon	Control	Immature embryos	Hypocotyls	Leaves
1	224,17	+	+	+	+	+	+	+	+	-
2	178,41	+	+	+	+	+	+	+	+	+
3	161,55	+	+	+	+	+	+	+	+	+
4	152,20	+	-	-	-	-	-	-	-	-
5	137,82	+	-	-	-	-	-	-	-	-
6	119,94	+	+	+	+	+	+	+	+	+
7	106,47	+	+	+	+	+	+	+	+	+
8	97,36	+	-	-	-	-	+	-	-	-
9	93,57	-	+	+	+	+	-	+	+	+
10	85,58	+	-	-	-	-	+	-	-	-
11	83,89	-	-	+	+	+	-	+	+	+
12	80,63	+	+	-	-	-	+	-	-	-
13	78,26	-	+	+	+	+	-	+	+	-
14	62,91	-	-	+	+	+	-	+	+	+
15	61,06	+	+	+	+	+	+	+	+	+
16	48,11	-	-	+	-	-	-	-	-	-
17	46,70	-	-	-	-	-	+	-	-	-
18	39,06	+	-	+	+	+	-	+	+	+
Total No. of bands		12	9	12	11	11	10	11	11	9

(+) appearance of band (-) disappearance of band

Table 6. Banding patterns of SDS-PAGE for water soluble protein fraction in R_2 generation of populations derived from the two sunflower entries through tissue culture of different explants.

Band number	Molecular weight (KDa)	Fig. (4)								
		Miak cv.					SP			
		Control	Immature embryos	Control	Immature embryos	Control	Immature embryos	Control	Immature embryos	Control
1	238.61	+	+	+	+	+	+	-	+	-
2	202.79	+	+	+	+	+	+	-	+	-
3	170.70	-	-	-	+	+	-	+	+	+
4	167.46	-	-	+	-	-	-	-	-	-
5	165.87	+	-	-	-	-	+	-	-	-
6	156.61	-	-	-	+	+	-	+	+	+
7	152.20	-	+	-	-	-	-	-	-	-
8	149.29	-	-	+	-	-	-	-	-	-
9	147.87	+	-	-	-	-	+	-	-	-
10	134.38	-	-	+	-	-	-	-	-	-
11	119.94	-	-	-	-	-	-	-	+	-
12	117.58	-	-	+	-	-	-	-	-	-
13	112.04	-	+	-	-	-	-	-	-	-
14	104.78	+	+	+	+	+	+	+	+	+
15	98.93	-	-	-	-	-	-	+	+	-
16	97.05	-	-	-	-	-	-	-	-	+
17	94.31	+	+	+	+	+	+	-	-	-
18	90.77	+	-	+	-	-	-	-	-	-
19	89.90	-	-	-	-	-	-	-	-	+
20	85.70	-	-	-	+	-	-	-	-	-
21	84.88	+	-	+	-	-	-	-	-	-
22	83.28	-	+	-	-	-	-	-	-	-
23	80.15	-	-	-	+	-	-	-	-	-
24	79.38	+	-	+	-	-	-	-	-	-
25	78.63	-	+	-	-	-	-	-	-	-
26	63.70	-	+	-	-	-	-	-	-	-
27	63.10	-	-	+	+	+	-	+	+	+
28	61.90	-	+	-	-	-	+	-	-	-
29	50.15	+	+	+	+	+	+	+	+	+
30	46.45	+	+	+	+	+	+	+	+	+
31	39.86	+	+	+	+	+	+	+	+	+
32	32.60	+	+	+	+	+	+	+	+	+
33	29.34	+	+	+	+	+	+	+	+	+
Total No. of bands		14	15	17	14	12	12	10	13	11

(+) appearance of band (-) disappearance of band

From the protein banding patterns and densitometric analysis of SDS-PAGE for water soluble protein fraction of sunflower populations in R_1 (Fig. 3 and Table 5), 18 bands were obtained with different molecular weights ranging from 224.17 to 39.06 KDa. The same result was obtained by Kasarada *et al* (1998). The lowest number of bands (9) in Miak cultivar was observed in the regenerated population derived from immature embryos explant. The highest number of bands (12) was also detected in the control of Miak cultivar and in the regenerated population derived from hypocotyls explant.

The most obvious feature was the disappearance of the (224.17 KDa) slow moving protein bands in the SP derived from leaves explant. Some bands were absent and could be considered as a somaclonal variation (152.20 and 137.82 KDa) in Miak populations. Some bands were polymorphic in both entries and the populations derived from different source of explants. The results of the present study indicate the presence of changes in protein patterns resulting from somaclonal variation induced through tissue culture of different explants *in vitro*. The fluctuation in protein patterns suggests the presence of variation in the genetic system (Brunel 1994, Anisimova *et al* 1995 and Jaramillo *et al* 1999).

Seed storage protein electrophoresis of the nine populations in R_2 generation is shown in Fig (4) and Table (6). From the protein banding patterns and densitometric analysis of SDS-PAGE for water soluble protein fraction of sunflower populations (Fig. 4 and Table 6) 33 bands were obtained with different molecular weights ranging from 238.61 to 29.34 KDa. The lowest number of bands (12) in Miak cultivar was detected in regenerated population derived from cotyledon explant. The highest number of bands (17) in Miak cultivar was observed in the regenerated population derived from hypocotyls explant. Thus, source of explant play a great role in the nature and magnitude of genetic changes in the chemical properties of the protein. In respect to the SP, the lowest number of bands (10) was observed in the regenerated population derived from immature embryos explant. The most obvious feature was the disappearance of the 238.61 KDa slow moving protein bands in the regenerated SP derived from immature embryo and leaf explants. Some bands were polymorphic in both entries and sources.

The overall results of the nine sunflower populations obtained by SDS-PAGE were in accordance with those obtained by the studied genetic parameters and were effective in studying the genetic background of the regenerated populations. Such conclusion was also reported by Wrigely *et al*

(1987), Rieseberg *et al* (1988), Abo El-Wafa and Ahmed (2001) and El-Fiki *et al* (2004). This technique was recommended to distinguish between cultivars and generating some biochemical genetic markers related to economically valuable characters. The appearance of new bands and absence of others was represented by different genotypes (Gepts 1990). Protein fingerprints of the progenies of *in vitro* regenerated sunflower plants revealed that there are great variations in band numbers and intensity. These findings clearly indicate that the induced somaclonal variations through tissue culture techniques could be useful in local breeding programs for both yield attributes and quality characters. Similar conclusion was reported by Rattray (1991).

The present data support the idea of inducing new genetic variation in the seed storage protein via the biotechnological methods. These new variation on the biochemical level could be helpful for the plant breeder to successfully improve the important trait or quality characters by selection in the derived populations. Anisimova *et al* (1995) reported that certain sunflower varieties could be differentiated by frequencies of individual bands. Moreover, Raymond *et al* (1991) found that the electrophoretic patterns of 11 S globulin (helianthinin) and albumin fractions were qualitatively and quantitatively similar in nine sunflower cultivars.

Magnitude of variation induced through tissue culture in sunflower depends on genotype and type of explant. In addition, plants produced from tissue culture were significantly superior in all studied growth characters as well as chemical constituents.

REFERENCES

Abo El-Wafa, A. and T. A. Ahmed (2001). Applications of molecular markers in crop improvement. Proceed. 2nd Pl. Breed. Conf. October 2nd, Assiut University. pp: 473-494.

Allard, R. W. (1999). Principles of plant breeding. 2nd ed. John Willey & Sons, Inc. New York. pp: 96-109.

Amer, I. M., Clara R. Azzam, A. A. Hob Allah and R. Shabana (2003). Role of genotype, explant, cultural conditions and their interactions in callus induction and plant regeneration from sunflower. Proceed. 3rd Pl. Breed. Conf., April, 26th, Giza. Egypt J. Plant Breed. 7(1): 195-212. Special Issue.

Anisimova, I. N., R. J. Fido, A. S. Tatham and P. R. Shewry (1995). Genotypic variation and polymorphism of 2S albumins of sunflower. Euphytica, 83(1): 15-23.

Azzam, Clara, R. (2000). Implication of conventional and biotechnological breeding approaches to improve sunflower cultivars and populations. Ph.D. Thesis, Fac. of Agric., Cairo University.

Azzam, Clara R., I. M. Amer, A. A. Hob Allah and R. Shabana (2003). Effect of auxin-cytokinin balance and explant on callus growth and regeneration in sunflower. Proceed. 3rd Pl. Breed. Conf., April, 26th, Giza. Egypt J. Plant Breed. 7(1): 213-228.Special Issue.

Baertlein, D. A. and R. G. McDaniel, (1987). Molecular divergence of alfalfa somaclones. Theor. Appl. Genet. 73: 575-580

Bajaj, Y. P. S. (1990). Somaclonal variation – origin, induction, cryopreservation, and implications in plant breeding. In: Bajaj, Y. P. S. (Ed) Biotechnology in Agriculture and Forestry. Somaclonal Variation in Crop improvement. I. pp: 3-48

Brunel, D. (1994). Denaturing gradient gel electrophoresis (DGGE) and direct sequencing of PCR amplified genomic DNA: rapid and reliable identification of *Helianthus annuus* L. cultivars. Seed Sci. and Technology 22(2): 185-194.

Coughlan, C. G., S. Frey, N. Hazebroek and J. C. Jennings (1998). New sunflower and soybean cultivars for novel vegetable oil types. Fett-Lipid. 100(4-5):177-181.

El-Fiki, A. A., A. I. H. Sayed and M. A. M. Moustafa (2004). Gamma radiation effects on growth and pattern of protein synthesis in organ and tissue cultures of sweet potato (*Ipomoea batatas* L. Lam). Arab J. of Nuclear Sci. and Applications, 37 (3): 253-263.

Evans, D. A. (1989). Somaclonal variation–genetic basis and breeding applications. Trends in Genetics 5: 46-50

Friedt, W. (1996). Use of biotechnology in sunflower breeding. Helia, 19(Special Issue): 161-179.

Gepts, P. (1990). The use of molecular and biochemical markers in crop evolution studies. Evolutionary Biol. 27: 51-94

Gosal, S. S., L. Vasiljevic and D. S. Brar (1988). Plant biotechnology and sunflower improvement. Proc. 12th Inter. Sunflower Conf., Inter. Sunflower Association, Novi Sad, Yugoslavia, 11: 599-609.

Greco, B., O. A. Tanzarella, G. Carrozzo and A. Blanco (1984). Callus induction and shoot regeneration in sunflower *(Helianthus annuus* L.*)*. Pl. Sci. 36: 73-77.

Jaramillo, S. G. N. O., O. J. Mediana, W. A. Rogriques, G. A. Tosello and P. Lopez (1999). Evaluation of some cereals, plants and tubers through protein composition. J. of Protein Chem. 18 (6):687-693.

Jones, L. H. (1988). The use of tissue culture in the improvement of oilcrops. Monograph British Plant Growth Regulator Group. 1988, No. 17, 37-55; in Plant lipids: targets for manipulation. Proceedings of a British Plant Growth Regulator Group meeting, London, UK.

Kasarada, D. D., K. M. Woodeard and A. E. Adalsteins (1998). Resolution of high molecular weight glutinin sub-units by a new SDS-PAGE system incorporating a neutral pH buffer. Cereal Chem. 75: 70-71.

Laemmli, U. K. (1970). Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature 227: 680-685.

Larkin, P. J. and W. R. Scowcroft (1981). Somaclonal variation - a noval source of variability from cell culture for plant improvement. Theor. Appl. Genet. 60: 197-214.

Larkin, P. J., P. M. Banks, R. Bhatti, I. S. Brettell, P. A. Davies, S. A. Ryan, W. R. Scowcroft, L. H. Spindler and G. J. Tamer (1989). From somatic variation to variant plants: mechanisms and applications. Genome 31: 705-711.

Marquard, R. (1987). Qualitats analytic in dienst der OL- pflanzen zuchtung. Fiett. Wissenschaft-Technologie 89: 95-99

McCoy, T. J. and E. T. Bingham (1977). Regeneration of haploid alfalfa plants from cells grown in suspension culture. Plant Sci. Lett. 10: 59-66.

Rattray, J. B. M. (1991). Plant biotechnology and the oils and fats industry. Biotechnol Plant Fats Oils (1991) 1-35

Raymond, J., V. Inquello and J. L. Azanza (1991). The seed proteins of sunflower: comparative studies of cultivars. Phytochemistry, 30(9): 2849-2856.

Rieseberg, L. H., D. E. Soltis and P. S. Soltis (1988). Genetic variation in *Helianthus annuus* and *H. bolanderi*. Biochemical Systematics and Ecology, 16(4): 393-399.

Santos, C. and G. Caldeira (1998). Callus formation and plant regeneration from protoplasts of sunflower calli and hypocotyls. Acta Societatis Botanicorum Poloniae. 67(1):31-36

Shabana, R. (1990). Performance of a new synthetic sunflower stock developed from local and introduced germplasm and further improvement via population improvement method. Helia 13(13): 11-16.

Steel, R. S. D. and J. H. Torrie (1980): Principles and Proceaures of Statistics: A biometrical approach 2nd ed. Mcgraw. Hill Book Co., New York, London.

Wrigely, C. W., I. L. Batey, W. P. Campbell and J. H. Skervitt (1987) Complementing traditional with novel procedures. Seed Sci. Technol., 15: 679-688.

Zorzoli, N. G., R. Mroginski and L. L. Picardi (1998). Cytoplasmic effects on the regeneration ability of sunflower. Pl. Breed. 117(2):188-190

التباين المستحدث عن طريق زراعة الأنسجة فى زهرة الشمس

كلارا رضا عزام

قسم بحوث الخلية-معهد بحوث المحاصيل الحقلية-مركز البحوث الزراعية-الجيزة

يعتبر محصول زهرة الشمس (دوار الشمس) من أهم المحاصيل الزيتية ذات الجودة العالية فى محتوى الزيت المستخلص منه، لذلك يهتم مربى النبات باستحداث تباينات وراثية من خلال طرق التربية المختلفة لتحسين المحصول ومكوناته وصفات الجودة ومنها المحتوى من الزيت وتتركز هذه الدراسة على تقييم التباين الناشئ خلال تقنيات زراعة الأنسجة (التباينات الجسمية) وعمل مقارنة بين نسل النباتات الناتجة من زراعة الأنسجة من أجزاء نباتية مختلفة: الأجنة غير الناضجة، الأوراق الفلقية، السويقة الجنينية والأوراق فى الجيلين الاستيلاديين الثانى والثالث فى تركيبين وراثيين مستخدمين هما الصنف ميك وعشيرة تركيبية. تمت زراعة البذور الناتجة من الجيل الاستيلادى الأول (R_0) للحصول على الجيل الاستيلادى الثانى (R_1) ثم تبعه الجيل الاستيلادى الثالث (R_3) فى موسمين متعاقبين فى الحقل بمركز البحوث الزراعية بالجيزة. وتم تقييم المحصول ومكونات المحصول ومحتوى الزيت والبروتين وعمل تفريد كهربائى للبروتينات الذائبة فى الماء. اختلفت متوسطات القيم للصفات المدروسة للصنفين المستخدمين وفقا للجزء النباتى المستخدم للاستيلاد. تفوقت النباتات الناتجة من زراعة الأنسجة (الناتجة عن طريق تباينات جسمية) فى معظم صفات المحصول ومكوناته ونسبة الزيت. الاختلافات كانت معنوية بالنسبة لطول النبات، عدد الأوراق/نبات وعدد البذور/نبات، وزن البذور/قرص ودليل البذرة ومحتوى الزيت لكلا الصنفين فى كلا الموسمين. زادت نسبة الزيت المئوية بشكل ملحوظ وكانت الزيادات ٣٩,٣ %، ٣٧,٦ %، ٣٦,٤ %، ٣٢,٠ % مقارنة بالكنترول للسلالات الناتجة من الأجنة غير الناضجة للمياك والعشيرة التركيبية فى الجيلين الاستيلاديين الأول والثانى على التوالى. تباين المدى ومعامل الاختلاف تبعاً للصفات المدروسة والأصناف والأجيال والجزء النباتى المستخدم. كما ظهر اعلى معدل للتباينات فى عدد ووزن بذور القرص فى الجيلين الاستيلاديين . وقد وجد ان النباتات الناتجة من زراعة الأنسجة تفوقت فى صفات النمو والمحصول ونسبة الزيت. ان زيادة معامل الاختلاف قد تؤدى الى انتخاب اكثر فاعلية فى الأجيال القادمة. وقد نجح استخدام تقنية SDS-PAGE للبروتينات الذائبة المستخلصة من بذرة دوار الشمس فى كل من الجيلين الاستيلاديين الثانى والثالث (لكل من الأجزاء النباتية المستخدمة) فى استنباط معلمات وراثية بيوكيميائية مرتبطة بالتباينات المختلفة الناشئة عن زراعة الانسجة، حيث ظهرت بعض الحزم البروتينية التى لم تتواجد فى المقارنة وغابت حزم بروتينية أخرى ذات أوزان جزيئية مختلفة. ومن النتائج يظهر بوضوح ان الاختلافات الناشئة عن طريق التباينات الجسمية المستحدثة خلال تقنية زراعة الأنسجة يمكن ان تكون مفيدة فى برامج التربية المحلية.

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