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ROLE OF GENOTYPE, EXPLANT, CULTURAL CONDITIONS AND THEIR INTERACTIONS IN CALLUS INDUCTION AND PLANT REGENERATION FROM SUNFLOWER

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ABSTRACT

To increase the regeneration ability in sunflower, different explants of seven genotypes were cultured under different environmental conditions. Callus induction from immature embryos, cotyledons and hypocotyls was 100% on media with sucrose less than 12% and tended to initiate somatic embryos. High sucrose concentration of 12% caused reduction in number of regenerated plants/immature embryo. The data indicated that the dark conditions at the first steps of culture is very important for regeneration, since it resulted in higher number of regenerated plants than light conditions. For plant regeneration protocol 2 was the best (3.7 plants/immature embryo) followed by protocol 1 (2.7 plants) and protocol 3 (2.6 plants) under dark conditions. Mean number of regenerated plants/immature embryo varied from one genotype to another. The highest number of regenerated plants was achieved by the synthetic population (2.4 plants/immature embryo) followed by HEL 226 (1.9 plants) and Line B (1.8 plants). While the lowest number of regenerated plants/immature embryo (0.6) was produced by the genotype HEL 179. These data suggest that plant regeneration from immature embryos of sunflower is dependent on the genotype.

Total number of regenerated plants induced from callus or somatic embryo derived from each cotyledon was generally higher under dark (9.3 plants) than under light conditions (7.9 plants) with all tested protocols except protocol No. 8. The highest number or regenerated plants/ cotyledon was produced with protocol 3 under both dark and light conditions (3.9 and 2.9 plants, respectively), followed by protocol 6 (1.9 and 1.6 plants, respectively). This suggests that high regeneration frequency could be obtained from calli derived from cotyledons cultured on MS medium supplemented by excess of KNO_3 (5 g L^{-1}). Generally, Syn. Pop.showed the highest regeneration (1.3 plants/ cotyledon) while HEL 179 was the lowest one (0.8 plants).

Protocol No. 3 was also the best for giving the highest number of regenerated plants/young hypocotyl under dark and light conditions (2.9 and

2.0 plants, respectively). The regeneration from hypocotyls under light was better than that under dark conditions using protocols 1, 2, 4, 7 and 8, while the opposite was true using protocols 3, 5 and 6. Syn. Pop. showed the highest number (2.2 plants), while HEL 226 showed the lowest (0.9 plants). Protocol 3 (consisted of HaR, HaR2, M1, S and R media) and hypocotyl explant are the best in obtaining the highest regeneration ability of calli. The synthetic population was the best genotype in regeneration ability from immature embryos, cotyledons and young hypocotyls.

Keywords: ***Sunflower, Helianthus annuus, Young hypocotyl, Cotyledon, Immature embryo, Dark and light conditions, Callus, Regeneration.***

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important oil crops of the world because its oil has a high unsaturated fatty acid and vitamin E (Gosal *et al* 1988). Its seed oil can be used for human consumption, as well as raw material for oleochemistry. It also can substitute mineral oil in various applications, such as fuels, lubricants or oils for hydraulic systems (Friedt 1996).

Tissue culture should be considered complementary to the classical breeding methods. The use of tissue culture technique by plant breeders is very valuable because the breeder is able to deal with the plants in a manner similar to that of microorganisms, the explant is converted to a large number of cells, which can be treated *in vitro* by different ways. Each cell in the callus or in the liquid media has a very good genetical potential if it is allowed to regenerate a new plantlet. Some of plantlets could have a new genetic make up although they all came from the same tissue; this what is called somaclonal variation. Such variation could be valuable to offer new genotypes with a quality or agronomic characteristic (Freyssinet and Freyssinet 1988). In many field crops such variation has been investigated and differences between plants were reported as indicated previously.

The ability to regenerate large numbers of plants from tissues cultures is a prerequisite for the successful application of this technology to crop improvement. Over the last years, a variety of techniques for regenerating sunflower by organogenesis (Greco *et al* 1984, Power 1987, Witrzens *et al* 1988, Espinasse *et al* 1989 and Azzam 2000) or somatic embryogenesis (Paterson and Everett 1985, Finer 1987, Freyssinet and Freyssinet 1988, Pelissier *et al* 1990 and Azzam 2000) has been reported. Plant regeneration by organogenesis from tissues of *Helianthus annuus*

remains, however, problematic. The regeneration frequency and the number of shoots produced per callus are highly variable, depending mainly on the genotype. For example, Greco *et al* (1984) reported callus induction and plant regeneration from only a single variety of sunflower, while Paterson and Everett (1985) described a method of regeneration limited to one cultivar of sunflower among hundred genotypes tested. Regeneration capacity is influenced by cultural conditions, genotype and their interactions (Sarrafi *et al* 1996)

Callus formation, shoot organogenesis of callus, and direct shoot production (embryogenesis) were dependent on the explant (shoot tips, stem nodes, hypocotyl segments, leaves, or cotyledon) as well as the medium composition and genotype (Greco *et al* 1984 and Paterson 1984).

Fertile plants can be regenerated from diverse explants through either organogenesis or somatic embryogenesis. Both morphogenic events can occur either directly from the explant or indirectly from a callus induced on the explant. Immature zygotic embryo often possesses a high morphogenic potential and it is frequently used for plant regeneration in species where regeneration from other explants is difficult. Somatic embryos or shoots can be induced *in vitro* on immature zygotic embryos of sunflower depending on the sucrose concentration of the culture medium (Charriere and Hahne, 1998).

Therefore, the experiments of this study were carried through culturing different immature zygotic parts of various genotypes on different media under various cultural conditions in order to maximize sunflower plant regeneration.

MATERIALS AND METHODS

The experiments of this investigation were carried out in the Molecular Cell biology Department, Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany.

Sunflower seeds of the commercial cultivar Miak (an open pollinated cultivar obtained from Oil Crops Research Section, Agricultural Research Center (ARC), Egypt), a synthetic population (Syn. Pop.) which is an open-pollinated population developed by the Agronomy Department, Faculty of Agriculture, Cairo University (Shabana 1990), B601022V (line B) which is a female line for the hybrid Hysun 333, and was obtained from Danton Company, Cairo, Egypt., the inbred line HA 300B (male fertile) which was obtained from Sun Gene company, Gatersleben, Germany, and different 3 accessions from Genebank of the Institute of Plant Genetics and

Crop Plant Research (IPK), Gatersleben, Germany i.e., HEL 179, HEL 212, HEL 226 were used as experimental materials.

Seeds of the above seven genotypes were planted and grown in an open field in the (IPK). The immature seeds of two different ages from pollination were discreted from heads and then were surface sterilized.

Immature zygotic embryos were dissected from immature seeds of the seven genotypes, 12 days after pollination (Fig. 1- Type A). While the immature seeds 15-21 days after pollination were used to dissect cotyledons and young hypocotyls (Fig. 1- Type B). The explants were cultured on 8 different kinds of media (Table 1) followed by transferring the tissue through a sequence of defined media using a specified timetable, i.e. eight protocols were used (Table 2).

The used explants were placed on the surface of different 8 solidified media in plastic petri dishes containing 25 ml medium (Table 2). Either from the immature embryos, cotyledons or young hypocotyls, 10 explants were placed per plastic petri dish using 8 replicates. Four replicates were incubated in the dark while the rest were kept under cool-white fluorescent light (35 μ Em^{-2} s^{-1}) 16/8h light (1500 lux) /dark cycle at a temperature of 25°C ±1°C for 3 weeks. Then the calli in the case of organogenesis pathway, with protocol No. 1 to 6 were transferred to the next media and kept under light conditions (16/8h).

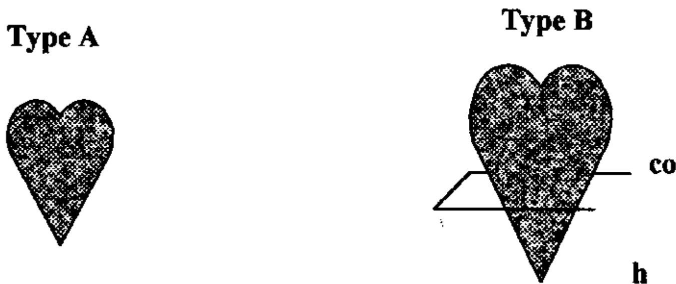


Fig. 1. The two types of immature embryos used in this experiment: Type A is the whole immature embryo dissected 12 days after pollination and Type B is the immature embryo dissected 15-21 days after pollination which was divided into 2 sections, i.e. immature cotyledon (co) and immature hypocotyl (h).

Table 1. Combinations of different culture media are used in this study (mg/l)

Contents	M1	M7	M10	HaR	HaR2	E1	E2	E3	E4	E5	S	R
Salts	MS	MS	MS	MS	MS	*MS basal	*MS basal	MS	*MS basal	*MS basal	MS	½ MS
Vitamins	MS	MS	MS	MS	MS	B5	B5	MS	B5	B5	MS	½ MS
KNO_3	-	-	-	5000	5000	-	-	-	-	-	-	-
Myo inositol	100	100	100	100	100	-	-	-	-	-	-	-
Adenine sulfate	-	-	-	40	40	-	-	-	-	-	-	-
Casamino acid	-	-	-	500	500	1000	-	-	-	1000	-	-
MES	-	-	-	-	-	500	-	-	-	500	-	-
2,4-D	-	-	-	-	-	-	1.0	-	1.0	-	-	-
NAA	-	0.1	0.5	1.0	-	-	-	-	-	-	-	0.5
6-BAP	0.5	2.0	1.0	1.0	1.0	1.0	-	1.0	-	1.0	-	-
GA_3	-	-	-	0.1	-	-	-	-	-	-	-	-
Sucrose (g.)	30	30	30	30	30	30	60	90	120	120	30	30
Agar (g.)	8	8	8	8	8	8	8	8	8	8	8	8
PH	5.8	5.8	5.8	5.5	5.5	5.8	5.7	5.0	5.7	5.8	5.8	5.8

*** MS Basal medium used was MS half-strength for the macro-nutrients and full-strength for the micro-nutrients.**

On the other hand, in the embryogenesis case (high sucrose concentration), with protocol nos.7 and 8, the somatic embryos were formed after approximately 3 weeks. After that the somatic embryos were transferred to the next fresh media and kept under the same dark conditions for 3 weeks to form the secondary somatic embryos. After that the secondary somatic embryos were transferred to the next medium to get shoots (shoots proliferation) under light conditions (16/8h).

Table 2. Experimental protocols of plantlet regeneration from different sunflower explants.

Main steps of the regeneration process	Experimental protocols							
	1	2	3	4	5	6	7	8
Callus induction + somatic embryos initiation	M7	M10	HaR	E1	E2	E3	E4	E5
Callus development + Secondary somatic embryos	M7	M10	HaR2	E1	E2	E3	E4	E5
Shoot proliferation	M1	M1	M1	M1	M1	M1	M1	M1
Shoot development	S	S	S	S	S	S	S	S
Rooting	R	R	R	R	R	R	R	R

The initiated shoots were transferred to S-medium, elongated shoots were then transferred to R-medium, and then they were allowed to grow up to plant maturity. The data were collected on no. of calli /explant, no. of somatic embryos/explant and no. of regenerated plants/explant.

RESULTS AND DISCUSSION

This experiment included different factors: 7 genotypes, 8 protocols, 3 explants and 2 lights conditions (dark and light). The main difference between the used protocols is the supplemented sucrose amount which was 3% in HaR, HaR2 and E1 media; 6% in E2 medium; 9% in E3 medium and 12% in E4 and E5 media.

Immature embryo explant

The data of Table (3) indicated that protocol 7 and protocol 8 (high sucrose concentration of 12 %) produced somatic embryos, while protocols from 1 to 6 which contain less than 120g L^{-1} sucrose initiated callus. On the other hand, high sucrose concentration of 12% in protocol 7 and 8 caused reduction in number of regenerated plants/immature embryo. Many investigators found also that 12% sucrose in media initiated the best somatic embryogenesis on immature embryos of sunflower (Chandler and Beard 1983, Finer 1987, Li *et al* 1988, Jeannin *et al* 1995 and Charriere and Hahne 1998). However, others produced somatic embryos on immature embryos by using high concentration of cytokinin (Encheva *et al* 1993 and Charriere and Hahne 1998).

The callus formation from 6 protocols (Nos.1-6) was 100% in all cases, but plant regeneration from protocol 2 was the best (3.7 plants/immature embryo) followed by protocol 1(2.7 plants / immature embryo) and protocol 3 (2.6 plants / immature embryo) under dark condition (Fig. 2). The callus induction and callus maintenance media of protocol 2 contained 0.5 mg L^{-1} NAA + 1.0 mg L^{-1} 6-BAP, protocol 1 contained 0.1mg L^{-1} NAA + 2.0mg L^{-1} 6-BAP, while protocol 3 contained MS supplemented by 5g L^{-1} KNO_3 +100mg L^{-1} Myo inositol + 1mg L^{-1} NAA and 6-BAP. With regard to genotype x medium interaction, Syn. Pop. showed the highest frequency of plant regeneration with protocol 4 (in the dark) which contained B5 vitamins +1g L^{-1} casamino acids + 1mg L^{-1} 6-BAP (Table 3) and healthy mature plants were recovered as shown in Fig. (3).

Table 3. **Mean number of calli (or somatic embryos) and regenerated plants (± SE) from each immature embryo of different sunflower genotypes, using protocols of different media compositions and light conditions.**

enotype	Experimental protocols*															
	1		2		3		4		5		6		7		8	
	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L
	No. of calli or somatic embryos (S) /immature embryo ± SE															
yn. Pop.	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	3.0 S ±1.3	2.8 S ±3.2	2.3 S ±1.5	2.5 S ±2.8
iak	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	3.0 S ±3.3	3.0 S ±2.6	2.3 S ±0.5	2.8 S ±1.5
ine B	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	2.8 S ±2.1	2.5 S ±2.0	2.8 S ±2.6	3.0 S ±2.8
a 300 B	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	3.0 S ±2.2	3.0 S ±1.4	3.0 S ±0.6	3.0 S ±1.4
EL 179	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	2.0 S ±1.5	1.9 S ±1.2	2.3 S ±1.9	2.4 S ±2.0
EL 212	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	3.0 S ±1.8	2.7 S ±2.2	3.0 S ±3.5	3.0 S ±2.6
EL 226	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	3.0 S ±1.1	2.9 S ±0.7	3.0 S ±2.0	2.4 S ±0.6

enotype	Experimental protocols*															
	1		2		3		4		5		6		7		8	
	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L
	No. of regenerated plants / immature embryo ± SE															
yn. Pop.	2.6 ±2.7	2.6 ±2.8	4.8 ±3.2	2.3 ±2.4	3.0 ±3.5	2.6 ±2.1	6.1 ±1.1	5.3 ±4.6	0.0 ±0.0	0.0 ±0.0	3.4 ±3.0	2.7 ±2.6	2.7 ±2.5	0.0 ±0.0	0.4 ±0.3	0.2 ±0.3
iak	2.2 ±1.4	2.0 ±1.2	2.1 ±1.6	1.7 ±0.5	3.2 ±1.3	2.6 ±1.8	0.4 ±0.1	0.4 ±0.3	0.0 ±0.0	0.0 ±0.0	0.5 ±0.4	0.2 ±0.4	0.0 ±0.0	0.0 ±0.0	0.7 ±0.3	0.1 ±0.3
ine B	4.2 ±3.2	2.3 ±1.2	5.3 ±4.0	1.6 ±0.8	2.7 ±2.0	1.5 ±1.3	0.9 ±0.2	3.0 ±1.9	0.0 ±0.0	0.0 ±0.0	3.5 ±1.6	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	1.3 ±1.1	1.8 ±0.9
a 300 B	4.7 ±3.3	0.6 ±0.3	5.5 ±3.8	1.1 ±0.8	0.8 ±0.5	0.3 ±0.4	0.1 ±0.3	0.3 ±0.5	0.0 ±0.0	0.0 ±0.0	0.8 ±1.2	0.5 ±0.8	0.5 ±0.6	0.3 ±0.8	0.7 ±1.0	0.4 ±0.9
EL 179	2.3 ±1.2	0.3 ±0.7	0.8 ±1.3	0.5 ±0.6	3.1 ±1.3	1.0 ±0.0	0.5 ±0.9	0.2 ±0.6	0.3 ±0.6	0.0 ±0.0	0.0 ±0.0	0.1 ±0.4	0.0 ±0.0	0.0 ±0.0	0.2 ±0.8	0.0 ±0.0
EL 212	1.3 ±2.3	0.0 ±0.0	3.9 ±2.7	0.0 ±0.0	1.4 ±0.5	1.6 ±0.9	1.9 ±2.3	5.1 ±3.6	0.0 ±0.0	0.0 ±0.0	2.2 ±1.6	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	1.7 ±0.3	3.0 ±5.2
EL 226	1.3 ±2.2	1.7 ±0.9	3.3 ±4.1	3.5 ±1.5	3.9 ±0.5	2.3 ±1.2	2.8 ±3.3	0.0 ±0.0	0.3 ±0.8	0.0 ±0.0	2.0 ±1.3	4.1 ±5.8	0.0 ±0.0	0.1 ±0.5	1.8 ±2.8	0.0 ±0.0

*** =For details of experimental protocols, see materials and methods (Tables 1 and 2).**
D = Dark conditions before shoot proliferation.
L = Light (1500 Lux) before shoot proliferation.
S = Somatic embryos.

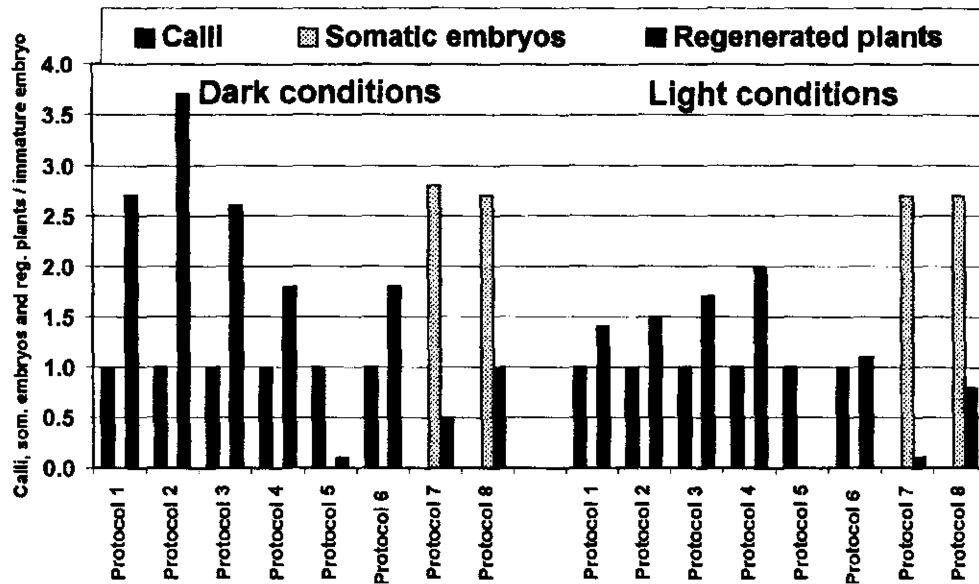
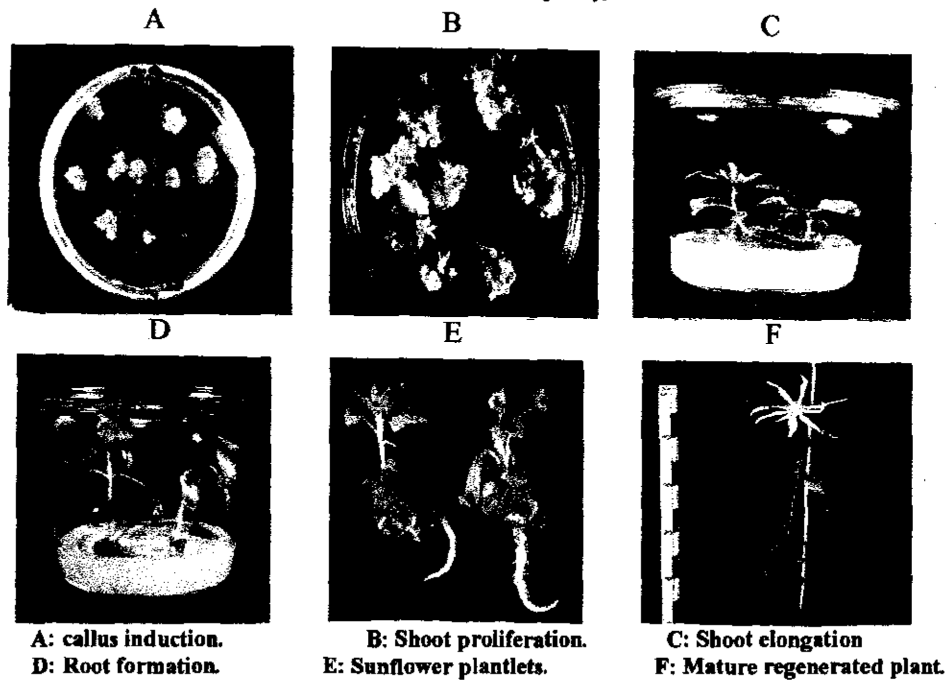


Fig. 2. Effects of Protocols of different media composition on callus or embryoid initiation from immature embryos and on plant regeneration under two light conditions over all the used sunflower genotypes.



A: callus induction. B: Shoot proliferation. C: Shoot elongation
D: Root formation. E: Sunflower plantlets. F: Mature regenerated plant.

Fig. 3. Main steps of the plant regeneration process from sunflower immature embryos.

Mean number of regenerated plants/immature embryo varied from one genotype to another (Table 3). The highest number of regenerated plants was achieved Syn. Pop. (2.4 plants/immature embryo) followed by HEL 226 (1.9 plants/immature embryo) and Line B (1.8 plants/immature embryo). The lowest number of regenerated plants/immature embryo (0.6) was produced by the genotype HEL 179. The data in Fig. (2) indicated that the dark conditions are very important for regeneration, since higher number of regenerated plants were obtained under dark than under light conditions.

These data suggest that plant regeneration from immature embryos of sunflower is dependent on the genotype. This conclusion is in agreement with that reported by Pelissier *et al* (1990), Power (1987), Espinasse and Lay (1989) and Jeannin and Hahne (1990).

Cotyledon explant

Effect of applying different protocols under two light conditions for culturing cotyledon on callus or embryoid initiation and plant regeneration of sunflower genotypes are illustrated in Table (4) and Fig. (4).

The callus induction from cotyledons for the all genotypes was 100% in the 6 protocols (Nos. 1- 6). However, protocol 7 and 8, which included media containing 12% sucrose initiated somatic embryos either under dark or light conditions. Frequency of somatic embryos, over all genotypes, was approximately equal in protocol 7 and 8. Meanwhile, the highest frequency (2.1 somatic embryos / explant) was noticed in protocol 8 (light) with HEL 226 (Table 4).

Also, Jeannin *et al* (1995) found that organogenesis occurred on media containing 120mM sucrose, while at 350 mM sucrose somatic embryos were observed. They added that the frequency of somatic embryos was increased with increasing sucrose concentration and vice versa for organogenesis from calli.

The data in Table (4) showed that the total number of plants regenerated from callus or somatic embryo derived from each cotyledon was generally higher under dark (9.3 plants) than under light conditions (7.9 plants) in all tested protocols except for protocol number 8, as shown in Fig. (4). Particulary contrast to our results, Paterson and Everret (1985) reported that hypocotyl and cotyledon explants grown in light were more responsive than those grown in dark. Development of embryos occured if cultures were exposed to light during the initial stages (Pelissier *et al* 1990).

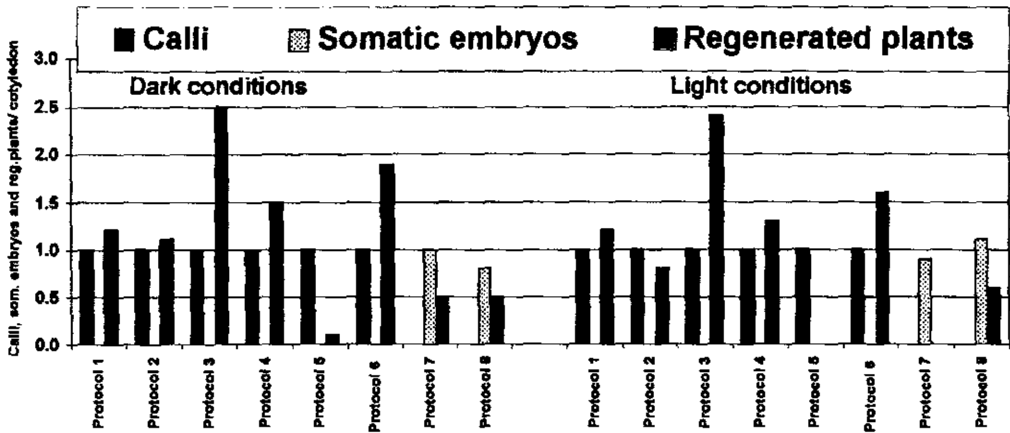


Fig. 4. Effects of protocols of different media compositions on callus or embryoid initiation from cotyledon and on plant regeneration under two light conditions over all the used sunflower genotypes

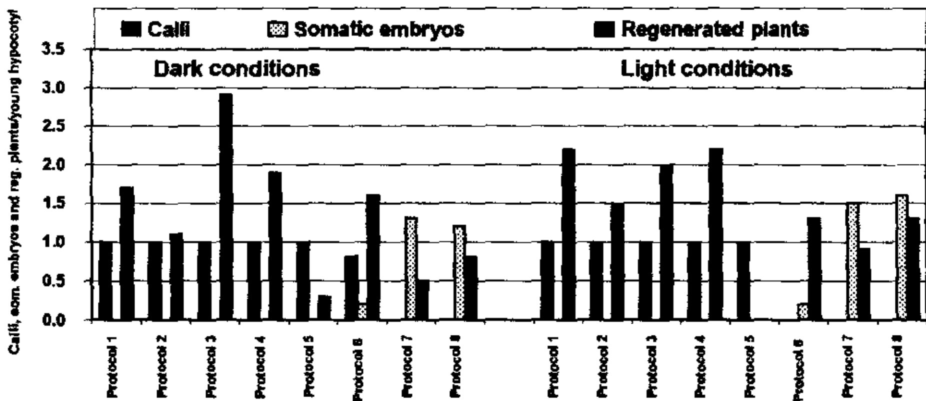


Fig. 5. Effects of protocols of different media compositions on callus or embryoid initiation from young hypocotyl and on plant regeneration under two light conditions over all the used sunflower genotypes

Table 4. **Mean number of calli (or somatic embryos) and regenerated plants (± SE) from each cotyledon of different genotypes, using protocols of different media composition and light conditions.**

enotype	Experimental protocols*															
	1		2		3		4		5		6		7		8	
	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L
	No. of calli or somatic embryos (S) /immature embryo ± SE															
yn. Pop.	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	0.9 S ±1.6	1.0 S ±2.5	1.0 S ±2.8	1.0 S ±0.0
Miak	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 S ±0.3	0.9 S ±0.3	0.2 S ±0.4	0.9 S ±1.2
Line B	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 S ±0.5	0.9 S ±1.1	1.0 S ±0.0	0.9 S ±0.5
HEL 179	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	0.9 S ±1.2	0.9 S ±0.5	0.9 S ±1.3	0.9 S ±0.6
HEL 212	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 S ±0.4	0.8 S ±1.1	0.9 S ±0.7	0.9 S ±0.3
HEL 226	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 S ±0.0	0.8 S ±1.5	1.0 S ±0.8	2.1 S ±2.3

enotype	Experimental protocols*															
	1		2		3		4		5		6		7		8	
	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L
	No. of regenerated plants / immature embryo ± SE															
yn. Pop.	1.4 ±2.8	0.0 ±0.0	0.9 ±1.6	0.1 ±0.4	4.9 ±2.3	4.1 ±3.3	0.4 ±0.5	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	4.0 ±3.4	4.0 ±2.1	0.3 ±0.5	0.0 ±0.0	0.1 ±0.3	0.0 ±0.0
Miak	1.0 ±0.3	1.6 ±2.1	2.2 ±1.3	2.0 ±1.8	1.7 ±2.5	1.5 ±0.6	1.2 ±0.8	2.4 ±1.5	0.0 ±0.0	0.0 ±0.0	1.9 ±1.2	2.3 ±2.0	0.0 ±0.0	0.0 ±0.0	0.6 ±0.8	0.5 ±0.7
Line B	3.0 ±1.3	1.9 ±0.9	0.2 ±0.4	0.0 ±0.0	3.9 ±1.8	2.9 ±1.6	0.0 ±0.0	0.8 ±0.6	0.0 ±0.0	0.0 ±0.0	1.6 ±2.4	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.7 ±0.8	1.1 ±0.8
HEL 179	0.0 ±0.0	0.5 ±1.2	2.3 ±1.7	1.5 ±1.3	1.9 ±2.3	2.5 ±0.8	1.3 ±0.9	0.0 ±0.0	0.3 ±0.4	0.0 ±0.0	1.5 ±0.9	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.7 ±1.5
HEL 212	0.0 ±0.0	2.0 ±1.3	0.0 ±0.0	0.0 ±0.0	1.7 ±1.2	1.3 ±2.5	6.1 ±2.6	1.4 ±0.7	0.2 ±0.3	0.0 ±0.0	2.6 ±2.1	1.7 ±0.8	1.4 ±0.9	0.0 ±0.0	0.0 ±0.0	0.5 ±0.3
HEL 226	1.9 ±2.1	1.4 ±1.1	0.7 ±0.6	1.3 ±0.9	0.7 ±1.2	2.0 ±1.5	0.1 ±0.2	3.4 ±2.8	0.1 ±0.3	0.0 ±0.0	0.0 ±0.0	1.3 ±0.4	1.0 ±1.2	0.0 ±0.0	1.7 ±1.5	0.9 ±0.6

*** =For details of experimental protocols, see materials and methods (Tables1and 2).**
D = Dark conditions before shoot proliferation.
L = Light (1500 Lux) before shoot proliferation.
S = Somatic embryos.

The highest number of regenerated plants/ cotyledon was produced with protocol 3 under dark and light conditions (3.9 and 2.9 plants, respectively) followed by protocol 6 (1.9 and 1.6 plants, respectively). This indicated that high frequency of promoting shoots through organogenesis pathway were obtained from calli cultured on MS media supplemented by

excess of KNO_3 (5g L^{-1}). Also, Paterson and Everett 1985, Lupi *et al* 1987 noticed that a combination of nitrogen sources such as NH_4^+, NO_3^-, and amino acids proved the optimal regeneration and cytokinin and auxin are generally required for shoot organogenesis.

On the other hand, protocol 5 showed no regeneration in dark and very low number of regenerated plants in light for HEL 179, HEL 212 and HEL 226 genotypes only (Table 4).

Genotypes exhibited differences in nos. of regenerated plants/ cotyledon. Generally, Syn. Pop.showed the highest regeneration (1.3 plants/ cotyledon), while HEL 179 was the lowest (0.8 plants/ cotyledon), as shown in Table (4).

Young hypocotyls explant

Effect of applying different protocols under dark and light conditions for culturing young hypocotyls on callus or somatic embryoid initiation and plant regeneration of different sunflower genotypes are presented in Table (5) and illustrated in Fig. (5).

The young hypocotyls of all used genotypes exhibited 100% callus induction for the protocols from 1 to 6 under both dark and light conditions. Protocol 7 and 8 which contained high sucrose concentration (12 %) across genotypes as well as protocol 6 for Syn. Pop., Miak and Line B under light conditions produced somatic embryos.These results are in agreement with those obtained by Pelissier *et al* (1990). The Syn. Pop. Genotype with protocol 7 under light conditions produced the highest number of somatic embryos/young hypocotyl (2.6) followed by Miak using protocol Paterson and Everett (1985) stated that hypocotyl and cotyledon explants grown in light were more responsive than those grown in darkness and Pelissier *et al* (1990) reported that development of somatic embryos occured if cultures were exposed to light during the initial stages.

The highest regeneration frequency derived from young hypocotyls was produced from cv. Miak using protocol number 3 under dark conditions (4.4 plantlets/young hypocotyl), followed by Syn. Pop.using protocol number 1 under the dark conditions (4.1 regenerated plants/young hypocotyl) and Line B using protocol number 4 under light conditions (4.1 regenerated plants/young hypocotyl explant), as shown in Table (5).

Protocol number 3 (which consist of HaR, HaR2, M1, S and R-media), produced the highest number of regenerated plants/young hypocotyl (2.9 and 2.0 plants under dark and light conditions, respectively) (Fig.5).

This may be due to the high nitrate concentration and presence of myo-inositol, casamino acids and adenine sulfate in media of this protocol. Also Paterson (1984) added 5g KNO_3 L^{-1} to MS medium for increasing the regeneration capacity of calli derived from sunflower hypocotyls.

The regeneration under light conditions was better than that under dark conditions using protocols nos. 1, 2, 4, 7, 8 and vise versa for protocols nos. 2, 3 and 6 which suggest some specific interaction between the protocols and light conditions (Fig. 5).

The tested genotypes showed differences in number of regenerated plants/young hypocotyl. In this regard, Syn. Pop. showed the highest number (2.2 plants), while HEL 226 exhibited the lowest (0.9 plants) as shown in Table (5). Lupi *et al* (1987) and Mohammad & Hassan (1988) obtained callus from hypocotyl cultured on medium containing different combinations of NAA and BA. While, Zezul *et al* (1995) induced embryoids from young hypocotyl and Freyssinet and Freyssinet (1988) regenerated plants from cultured young hypocotyls on MS or B5 media. On the other hand, Robinson and Adams (1987) found that ethylene production was not correlated with light conditions (light - dark) but seemed to be due to a difference in sensitivity to ethylene at a specific time during the growth of sunflower hypocotyl. From these findings, it is suggested that response genotype differences in response may be due to different endogenous hormone levels and/or different sensitivities to hormones (Robinson *et al* 1987).

From this investigation, it is concluded that protocol 3 was able to give the highest plant regeneration frequency from all tested explants, i.e., immature embryos, cotyledons and hypocotyls. Therefore, the best procedure for plant regeneration from immature embryos, cotyledon and hypocotyl explants of sunflower would be as follows: callus induction on HaR medium, callus propagation on HaR2 medium, shoot proliferation on M1 medium, shoot development on S-medium and rooting on R-medium (the composition of each medium is illustrated in Materials and Methods). Moreover, Syn. Pop. was the best in showing the highest regeneration capacity from calli derived from all tested explants. The best results of plant regeneration were generally obtained from calli derived from hypocotyls.

Table 5. Mean number of calli (or somatic embryos) and regenerated plants (± SE) from each young hypocotyl of different genotypes, using protocols of different media composition and light conditions.

enotype	Experimental protocols*															
	1		2		3		4		5		6		7		8	
	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L
	No. of calli or somatic embryos (S) /immature embryo ± SE															
yn. Pop.	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	0.2 S ±0.8	1.9 S ±1.4	2.6 S ±2.1	1.8 S ±2.5	2.5 S ±2.2
Miak	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	0.6 S ±1.4	1.8 S ±1.1	2.3 S ±0.7	1.4 S ±0.5	2.6 S ±1.1
Line B	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	0.3 S ±0.4	1.3 S ±0.9	1.3 S ±0.5	1.2 S ±1.0	1.2 S ±0.7
HEL 179	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.1 S ±0.8	1.1 S ±0.4	0.7 S ±1.3	1.4 S ±0.7
HEL 212	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	0.9 S ±0.6	0.8 S ±0.2	0.9 S ±0.2	0.9 S ±0.4
HEL 226	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	1.0 ±0.0	0.8 S ±0.2	0.9 S ±0.5	1.0 S ±1.0	1.0 S ±0.7

enotype	Experimental protocols*															
	1		2		3		4		5		6		7		8	
	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L
	No. of regenerated plants / immature embryo ± SE															
yn. Pop.	4.1 ±3.8	4.0 ±3.1	1.9 ±1.5	1.8 ±2.1	3.9 ±2.5	3.9 ±2.4	2.9 ±3.1	2.6 ±3.2	0.0 ±0.0	0.0 ±0.0	3.3 ±3.2	2.0 ±2.4	0.5 ±0.7	1.4 ±1.1	1.5 ±0.8	1.8 ±1.0
Miak	3.9 ±1.4	0.9 ±0.3	1.5 ±0.6	2.0 ±1.3	4.4 ±0.3	1.4 ±0.9	0.4 ±0.5	0.8 ±0.6	0.0 ±0.0	0.0 ±0.0	1.3 ±0.8	0.4 ±0.7	0.8 ±0.3	1.9 ±1.5	1.2 ±0.8	1.8 ±0.9
Line B	1.0 ±0.0	3.5 ±2.3	0.9 ±0.4	2.8 ±1.5	1.8 ±0.6	2.1 ±1.6	1.4 ±0.9	4.1 ±2.4	0.0 ±0.0	0.0 ±0.0	2.4 ±1.3	0.0 ±0.0	1.0 ±0.4	1.2 ±0.9	0.3 ±0.4	0.7 ±1.3
HEL 179	0.0 ±0.0	2.8 ±0.3	2.2 ±0.2	0.0 ±0.0	2.3 ±0.7	2.3 ±0.2	1.5 ±0.4	2.8 ±0.4	0.1 ±0.1	0.0 ±0.0	0.0 ±0.0	2.2 ±0.1	0.6 ±0.2	0.9 ±0.2	0.3 ±0.3	1.2 ±0.5
HEL 212	0.0 ±0.0	1.2 ±0.5	0.0 ±0.0	2.2 ±0.9	1.9 ±0.4	0.0 ±0.0	3.9 ±1.3	1.8 ±0.3	1.7 ±1.2	0.0 ±0.0	2.7 ±0.3	2.6 ±0.7	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.6 ±1.1
HEL 226	1.2 ±0.6	0.9 ±0.2	0.0 ±0.0	0.2 ±0.2	2.9 ±0.7	2.5 ±0.4	1.2 ±0.3	0.9 ±0.3	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.6 ±0.5	0.0 ±0.0	0.1 ±0.3	1.4 ±0.3	1.7 ±1.0

*** =For details of experimental protocols, see materials and methods (Tables1and 2).**
D = Dark conditions before shoot proliferation.
L = Light (1500 Lux) before shoot proliferation.
S = Somatic embryos.

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دور التركيب الوراثي والجزء النباتي وظروف مزارع الانسجة على إستحداث الكالوس وإستيلاد نباتات زهرة الشمس .

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لـزيادة القـدرة على إستيلاد نباتات من أجزاء مختلفة من نبات زهرة الشمس . تم زراعة الأجـنة غير الناضجة، والفلقات غير الناضجة و السويقة الجنينية السفلى غير الناضجة لسبعة أصناف مخـتلفة التركيـب الوراثـي مـن نبات زهرة الشمس على ثمانية بيئات مختلفة ثم استخدمت ثمانية بروتوكولات لتحديد البيئة المناسبة لكل تركيب وراثي لاستحداث الكالوس وإنتاج الأجنة الجسمية وتحديد ظـروف الإضـاءة اللازمة لذلك. وقد وجد أن استحداث الكالوس من الأجزاء النباتية المستخدمة في الدراسـة كان ١٠٠% باستخدام ستة بروتوكولات (١– ٦). بينما بروتوكول ٧ و ٨ (المحتوى على ١٢% سكروز) أعطت أجنة جسمية، و أدت لنقص فى عدد النباتات المستولدة. و دلت النتائج على أن تعرض مزارع الأنسجة لفترة من الظلام مهم لإستيلاد النباتات. وكان عدد النباتات الناتجة من الكالوس الناشـئ تحت ظروف الظلام اكثر من الكالوس الناتج تحت ظـروف الإضاءة. وبالنسبة لعدد النباتات المسـتولـدة كان البروتوكـول رقم ٢ افضل حيث أعطى ٣,٧ نبات/جنين غير ناضج. تلاه بروتوكول رقم ١(٢,٧ نبات/جنين غير ناضج) و بروتوكول ٣ (٢,٦ نبات/جنين غير ناضج) في ظروف الظلام. متوسط عدد النباتات المستولدة من كل جنين غير ناضج اختلفت من تركيب وراثي إلى آخر ، وكان أعلى عدد تم إستيلاده من العشيرة التركيبية (٢,٤ نبات/جنين غير ناضج) تلاها الصنف HEL 226 (١,٩ نـبات) والسلالة ب (١,٨ نبات/جنين غير ناضج) بينما اقل عدد من النباتات/جنين غير ناضج نتج من

الصــنف HEL 179 (٠,٦ نبات/جنين غير ناضج) ويتضح من ذلك أن عدد النباتات المستولدة يعتمد على التركيب الوراثي لنبات زهرة الشمس.

عــدد النــباتات المستولدة الكلية من الكالوس الناشئ من الفلقات غير الناضجة كان عموما عالـــي في ظروف الظلام (٩,٣ نبات/فلقة غير الناضجة) اكثر من ظروف الإضاءة التي كانت ٩,٧نبات لكــل البروتوكـــولات المستخدمة ماعدا بروتوكول رقم ٨. نتج أكبر عدد للنباتات المستولدة لكل فلقة غير ناضجة من بروتوكول رقم ٣ تحت ظــــروف الظلام و الإضاءة (٢,٥، ٢,٤ نبات, على التوالي) يلــيه بـروتوكول ٦ (١,٩، ١,٦، على التوالي) . وهذا دليل على انه يمكن الحـــصول على معــدل عــالي من النباتات من الكالــوس الناتج من الفلقتين غير الناضجتين والمنزرع على بيئة MS مضاف لهــا كمــية كبـيرة من نترات البوتاسيوم (٥جم/لتر). وكان تأثير التركيب الوراثي على عدد النباتات المعــستولدة واضح. عموما أظهر الصنف التركيبي اظهر أعلي معدل استيلاد للنباتات (١,٣ نبات/جزء) بينما HEL 179 كان الأقل (٠,٨ نبات/جزء).

كــان بـروتوكول رقــم ٣ افضل في إعطاء أعلى عدد من النباتات المستولدة من السويقة الجنينــية غــير الناضجة تحت ظروف الظلام والإضاءة (٢,٩، ٢,٠ نبات/جزء, على التوالي). بينما الاستيلاد من الكالوس الناشىء تحت ظروف الإضاءة كان افضل من الظلام تحت ظروف بروتوكول رقم ١، ٢، ٤، ٧ و ٨. والعكــس فــي بــروتوكول رقــم ٣، ٥ و ٦. و مرة أخرى اختلفت الأصناف في اســتجابتها للاستيلاد، وكان افضل الأصناف هي العشيرة المركبة التي أنتجت (٢،٢ نبات/جزء نباتي)، بيـــنما HEL226 كان الأقل إنتاجا للنباتات (٠,٩ نبات/جزء نباتي). بروتوكول رقم ٣ الذي تكون من HaR, HaR2, M1, S and R-media مــع السويقة الجنينية السفلي كان الأفضل للحصول على نباتات. والصنف التركيبي كان افضل الأصناف للحصول على نباتات من الجنين غير الناضج و الفلقات غـــير الناضـــجة والسويقة الجنينية السفلي غير الناضجة. مع وجود تفاعل واضح بين كلا من تركيب البيـــئة وظروفها والتركيب الوراثي والجزء النباتي المستخدم في مزارع الانسجة. و بصفة عامة كانت أفضـــل الأصـــناف هـــي العشـــيرة التركيبـــية وأفضل جزء نباتي هو السويقة الجنينية السفلي وأفضل بروتوكول رقم ٣ بالنسبة للاستيلاد في عباد الشمس.

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