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Effect of genotype and various hormonal combinations on regeneration of sunflower (*Helianthus annuus* L.)

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Abstract

In this study, the effect of cotyledon and hypocotyl explants of five sunflower genotypes (Azargol, Farokh, Maste, CIRENA and ESBIBA) were studied on different hormonal combinations IAA, NAA, BAP, KIN and 2,4-D on regeneration of Sunflower. Variance analysis results showed that evaluated cultivars had significant differences in response to different hormonal combinations. The results of the mean comparison showed that maximum amount of callus was obtained in hypocotyl explant, Farokh genotype in all media. Maximum number of airbud was observed in cotyledon explants of Master and ESBIBA genotypes in medium which was containing hormonal combination (IAA+BAP) as well as, ESBIBA genotype in medium which was containing hormonal combination (IAA+KIN). Also maximum amount of rooting were related to hypocotyl explants of Azargol and ESBIBA genotypes, in media which were containing hormonal combination (NAA+KIN) as well as CIRENA genotype in medium which was containing hormonal combination (NAA+BAP).

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Introduction

Sunflower (*Helianthus annuus* L.) is the fourth most important oil crops in the world. The technology for processing sunflower-oil into biodiesel-oil has been developed lately, and as a result; the necessity of good quality sunflower oil is increasing rapidly (Ikeda *et al.,* 2005). Biotechnological techniques such as tissue culture and gene transfer systems have been used for improvement of sunflower, but these techniques are mainly limited by the tissue culture response of commercial varieties (Nestares *et al.,* 2002). The regeneration ability of sunflower from different parts of plant material has been investigated by several authors, such as Greco *et al.,* 1984; Paterson and Everett, 1985. In different explants of *Helianthus*, cotyledons and young hypocotyls are advantageous since they are easily and quickly available and posses a high potential for direct and indirect ways of regeneration (Ozyigit *et al.,* 2002). Organogenic regeneration from sunflower cotyledons have been reported by several laboratories (Knittel *et al.,* 1991; Ceriani *et al.,* 1992; Nestares *et al.,* 1996; Sarrafi *et al.,* 1996; Baker *et al.*, 1999). Fabijan *et al.,* (1981b) reported the effects of the brief application of various growth regulators on the production of adventitious root primordia in sunflower hypocotyls. Many reports showed that for *Helianthus* genus, 1 mg/L 2,4-D is convenient for callus formation in both hypocotyl and cotyledon explants and 1 mg/L BA and 0.5 mg/L NAA together are also convenient for shoot regeneration (Ozyigit *et al.,* 2002, 2006). The aim of this study was to evaluate regeneration efficiency from cotyledon and hypocotyl explants of five sunflower genotypes.

Materials and methods

In order to study the effect of genotype and various hormonal combinations on regeneration of five sunflower genotypes including "Azargol", "Farokh", "Maste", "CIRENA" and "ESBIBA", an experiment was conducted in a randomized complete block design with three replicates at the plant tissue laboratory of the College of Agriculture, Urmia University, Iran in October, 2012. Before culturing, at first the seeds were sterilized by immersion in ethanol (70%) for 60 s, then in 1.25% aqueous solution of sodium hypochlorite for five minute. This was followed by rinsing in sterilized distilled water at least three times. Seeds were germinated on hormone free MS (Murashige and Skoog, 1962) medium. The medium contained MS vitamin solution, basal salt mixture, 30 g sucrose and 7 g agar (Sigma Chemical Co.). Seeds were kept at growth chamber with specific photoperiod conditions including 16 hour light (6000 lx) and 8 hour darkness, at 25±2 ^{o}C.Hypocotyls and cotyledons explants were taken from seedlings two days after culturing, then these explants were cultured on Murashige and Skoog (MS) medium contained 8 g agar and were supplemented with different combination of hormones IAA, NAA, BAP, KIN and 2,4-D in 60mm diameter Petri dishes. The media pH was adjusted to 5.8 before autoclaving. The explants were cultured in a growth chamber after 28 days and percentage of regeneration was evaluated. The number of roots, shoots and calli of regenerated explants were measured.The data were analyzed by MSTATC software, Duncan's multiple range test (at the 0.05 and 0.01 probability levels) was employed for the mean comparisons. EXCEL and Word 2013 were used to draw diagrams and tables.

Results and discussion

In sunflower, reports are available on shoot regeneration from hypocotyls, cotyledons, leaves and meristematic tissues of young plantlets (Sujatha and Prabakaran, 2001). Furthermore, regeneration quality was either low with abnormal morphogenesis, frequency by organogenesis essentially depended on genotype and its interaction with culture conditions (Ozyigit *et al.,* 2006). The results of variance analysis (Table 3) showed that the cultivars, explants and hormonal compounds among main factors were significantly different ($P \leq 0.01$) based on the most studied traits and significant genetic diversity was observed for parameters of organs regeneration. So, factors of hormonal combinations and explant types of all traits and factors of genotype such as rooting, grain refining, callusing, and the number of root+callus were significantly different within probability level 1% (Table 3). Existence of significant differences between genotypes was accordance with

the results of Zhang and Bahala (1999) on rapeseed. Statistical analysis showed that triple interactive effects were not significant for the trait callusing; therefore, the effects of combination were evaluated. All dual effects had significant difference in the 1% probability level for the callusing (Table 3). Genetic diversity was reported earlier in response to the cultivation of sunflower cotyledon by Power, (1987), Berrios *et al.* (1999), Sarrafi *et al.* (1996), Espinasse *et al.* (1989), Mayor *et al.* (2003) and Azadi *et al.* (2002).

Table 1. Names of studied genotypes.

No. of genotype	Name of genotype
1	Azar Gol
2	Farokh
3	Master
4	CIRENA
5	ESBIBA

Callus is an unorganized mass of plant cells and its formation is controlled by growth regulating substances present in the medium (auxins and cytokinins) (Shah *et al.,* 2003). The specific concentration of plant regulators needed to induce callus formation, varies from species to species and can even depend on the source of explant (Charriere *et al.,* 1999). Furthermore, genotype is one of the most important factors for callus induction like shoot regeneration in tissue culture studies (Sarrafi *et al.,* 1996; Punia and Bohorova, 1992). In many tissue culture studies like sunflower, callus can be obtained from different explants with various plant hormones and hormone combinations (Ozyigit *et al.,* 2006; Shah *et al.,* 2003). The parts of hypocotyls and cotyledon explants had a strong tendency to form callus on the used different hormonal treatments and after two weeks, callus formation was observed on the components in many of the used culture media (Figure 1). The results of the mean comparison were obtained from dual effects for callusing showed that in the interaction between genotype and hormonal combination the maximum amount were related to Farokh genotype in the media which were containing hormonal substances (NAA+BAP), (NAA+KIN) and (2,4-D+BAP) (Table 4), in the interaction between hormonal combination and explants the maximum amount were related to hypocotyl explants in the media which were containing hormonal combinations (NAA+BAP) and (NAA+KIN) (Table 5), in the interaction between genotype and explant the maximum amount was obtained from Farokh genotype in the medium which was containing hormonal combination (NAA+KIN) (Table 6). The results in the this study showed that callus formation was not only influenced by cytokinin. Similar results was reported by Hasani *et al.,* (2008). Among of two auxin IAA and NAA, NAA hormone was acted more effective in producing of callus, this results had attunement with the results of Baskaran ,2006. Ceriani *et al.* (1992) who used 20 different genotypes and did not get any regeneration for 9 of them, but the best regeneration was obtained on MS medium, which contains 1 mg/l BA and 0.75 mg/l NAA (90 %) (Ceriani *et al.,* 1992). As it can be seen from the studies above, especially in sunflower tissue cultures, hypocotyls and cotyledon explants are good regeneration materials that show different regenerative behavior when kept in a culture, depending on their genotype.

Table 2. Number and type of regulatory compounds.

No. of combination	Hormonal combinatio
1	NAA + BAP
2	NAA + KIN
3	IAA + BAP
4	IAA + KIN
5	2,4-D + BAP
6	2,4-D + KIN

After two weeks, the emergence of different hormonal treatments was clearly visible on explants parts, over time, the roots number and size were added (Figure 2). It should be noted that rooting phenomenon in sunflower explants were hardly, or not observed at all in some genotype varieties. The results of the mean comparison for rooting indices showed that effect of different hormonal combinations which were containing an auxin and a cytokinin, were significant on the rooting. The maximum amount of rooting in cotyledon explants was related to Azargol genotype in medium which was containing hormonal combination (NAA+KIN), and the least amount was found in

medium which was containing hormonal combination (IAA+KIN) and genotypes of Master and Azargol (Table 7). The maximum amount of rooting indices in hypocotyl explant was related to ESBIBA and Azargol genotypes in the medium which was containing hormonal combination (NAA+KIN) and CIRENA genotype in the medium which was containing hormonal combination (NAA+BAP). The lowest value of this indices were observed in Azargol genotype in medium which was containing hormonal combination (IAA+BAP) and ESBIBA genotype in medium which was containing hormonal combination (IAA+KIN). The rooting for different genotypes were not observed in some of hormonal combinations (Table 8).

Table 3. Variance analysis the effect of genotype and growth regulators on the different characteristics in regeneration of sunflower.

		Mean-square						
Sources of variation	Df	Root	Airbud	Callus	Root+Airbud	Root+Callus	Callus+Airbud	Root+Callus+Airbud
Explant	1	0.043**	17.580**	10.023**	0.579**	0.015**	1.633**	0.239**
Genotype	4	0.656**	0.492**	2.229**	0.029^{ns}	0.602**	0.070^{ns}	0.023^{ns}
Explant × Genotype	4	0.066^{ns}	0.731**	0.664**	0.054^{ns}	0.073**	0.168**	0.048*
Hormonal combination	5	5.079**	1.651**	2.160**	0.457**	4.364**	0.333**	0.254**
Hormonal combination × Explant	5	0.090^{ns}	1.243**	1.919**	0.243**	0.044**	0.261**	0.106**
Hormonal combination × Genotype	20	0.549**	0.082**	0.498**	0.042*	0.487**	0.086**	0.032*
Hormonal combination × Genotype × Explant	20	0.170**	0.081**	0.175^{ns}	0.068**	0.197**	0.086**	0.036*
Error	180	0.053	0.039	0.122	0.023	0.048	0.039	0.020
%cv		24.42	19.56	20.70	19.79	23.93	24.18	18.76

*,** and ^{ns} significant at 5% and 1% and non-significan, respectively.

Table 4. Mean comparison of callus for interaction the genotypes × hormonal combination.

Genotype	Hormonal combination	Callus
Azar Gol	NAA + BAP	3.5 abc
Azar Gol	NAA + KIN	3.5 abc
Azar Gol	IAA + BAP	2.25 bcdefg
Azar Gol	IAA + KIN	1.75 efg
Azar Gol	2,4-D + BAP	3 abcde
Azar Gol	2,4-D + KIN	3.25 abcde
Farokh	NAA + BAP	4 a
Farokh	NAA + KIN	4 a
Farokh	IAA + BAP	3.38 abcd
Farokh	IAA + KIN	3 abcde
Farokh	2,4-D + BAP	4 a
Farokh	2,4-D + KIN	3.75ab
Master	NAA + BAP	3.38 abcd
Master	NAA + KIN	3 abcde
Master	IAA + BAP	2 cdefg
Master	IAA + KIN	1.13 fg
Master	2,4-D + BAP	1.88 defg
Master	2,4-D + KIN	0.88 g
CIRENA	NAA + BAP	3.88 a
CIRENA	NAA + KIN	3.5 abc
CIRENA	IAA + BAP	2.25 bcdefg
CIRENA	IAA + KIN	1.25 fg
CIRENA	2,4-D + BAP	0.88 g
CIRENA	2,4-D + KIN	2.63 abcdef
ESBIBA	NAA + BAP	2.13 cdefg
ESBIBA	NAA + KIN	2.63 abcdef
ESBIBA	IAA + BAP	1.75 efg
ESBIBA	IAA + KIN	0.88g
ESBIBA	2,4-D + BAP	3.75 ab
ESBIBA	2,4-D + KIN	3 abcde

Table 5. Mean comparison of callus for interaction explant × hormonal combination.

Explant	Hormonal combination	Callus
Cotyledon	NAA + BAP	2.9 b
Cotyledon	NAA + KIN	2.8 b
Cotyledon	IAA + BAP	0.9 c
Cotyledon	IAA + KIN	0.4 c
Cotyledon	2,4-D + BAP	2.8 b
Cotyledon	2,4-D + KIN	2.65 b
Hypocotyl	NAA + BAP	3.85 a
Hypocotyl	NAA + KIN	3.85 a
Hypocotyl	IAA + BAP	3.75 a
Hypocotyl	IAA + KIN	2.8 b
Hypocotyl	2,4-D + BAP	2.6 b
Hypocotyl	2,4-D + KIN	2.75 b

Table 6. Mean comparison of callus for interaction the genotype × explants.

Genotype	Explant	Callus
Azar Gol	Cotyledon	2.04 cd
Farokh	Cotyledon	3.38 ab
Master	Cotyledon	1.34 d
CIRENA	Cotyledon	2.21c
ESBIBA	Cotyledon	1.42 d
Azar Gol	Cotyledon	3.71 a
Farokh	Hypocotyl	4 a
Master	Hypocotyl	2.75 bc
CIRENA	Hypocotyl	2.59 bc
ESBIBA	Hypocotyl	3.3 ba

The results revealed that hormonal combinations (NAA+BAP) and (NAA+KIN) would lead to production of adventitious roots in the basal medium MS. This finding shows the effect of dominance of auxin (NAA) relative to cytokinin (BAP and KIN) in the rooting. Hashemi Abadi and Kaviani (2008), and Velcheva *et al.,* (2005) in two separate researchs concluded that adding NAA to the culture media increased the number of roots in the Aloe vera that was similar to the survey results (Hashemiabadi and Kaviani, 2008 ; Velcheva *et al.,* 2005). Contrary to results which were obtained from this study Evans and Harangozo, (1982) mentioned the existence of IAA in the medium affects morphogenesis of root. In the research conducted on chicory by Vuylsteker *et al.,* in 1997 has been refered to better impact and more desirable IAA than NAA in creating of the lateral root, this mismatch was mainly due to differences in using cultivars. After two weeks of emerging, shoots were appeared on cotyledon pieces in media which were contained hormonal substances (NAA+BAP), (NAA+KIN), (IAA+BAP) and (IAA+KIN). The produced buds had grown considerably and leaves had developed at the end of the third and fourth weeks (Figure 3). On the other hand, the direct shoot formation of hypocotyl explants were been barely. So, we observed a lack of shoot formation in the most of hormonal compounds. The results of the mean comparison airbud trait related to cotyledon explant showed that the highest average number of airbud existed in medium which was containing hormonal combination (IAA+BAP) in genotypes of Master and ESBIBA as well as in medium which was containing hormonal combination (IAA+KIN) in ESBIBA genotype. Also the minimum amount for this trait was obtained in medium which was containing hormonal combination (NAA+BAP) in genotype Farokh (Table 7).

Table 7. Mean comparison of characteristics for interaction between genotypes and hormonal combination in cotyledon explant.

Genotype	Hormonal combination	Root	Airbud
Azar Gol	NAA + BAP	2.25ab	1efg
Azar Gol	NAA + KIN	3.5a	1efg
Azar Gol	IAA + BAP	0.01d	2.25abc
Azar Gol	IAA + KIN	0.25d	1.75bcde
Azar Gol	2,4-D + BAP	0.01d	0.01h
Azar Gol	2,4-D + KIN	0.01d	0.01h
Farokh	NAA + BAP	0.01d	0.25gh
Farokh	NAA + KIN	2b	0.75efgh
Farokh	IAA + BAP	0.01d	0.5fgh
Farokh	IAA + KIN	0.01d	2abcd
Farokh	2,4-D + BAP	0.01d	0.01h
Farokh	2,4-D + KIN	0.01d	0.01h
Master	NAA + BAP	0.75cd	1.5bcde
Master	NAA + KIN	2.5ab	2abcd
Master	IAA + BAP	0.01d	3.25a
Master	IAA + KIN	0.25d	2.5abc
Master	2,4-D + BAP	0.01d	0.01h
Master	2,4-D + KIN	0.01d	0.01h
CIRENA	NAA + BAP	1.5bc	2.25abc
CIRENA	NAA + KIN	2ab	2abcd
CIRENA	IAA + BAP	0.01d	2abcd
CIRENA	IAA + KIN	0.01d	2.5abc
CIRENA	2,4-D + BAP	0.01d	0.01h
CIRENA	2,4-D + KIN	0.01d	1.25cdef
ESBIBA	NAA + BAP	0.01d	2.25abcd
ESBIBA	NAA + KIN	2.5ab	2.75ab
ESBIBA	IAA + BAP	0.01d	3.25a
ESBIBA	IAA + KIN	0.01d	3.25a
ESBIBA	2,4-D + BAP	0.75cd	0.5fgh
ESBIBA	2,4-D + KIN	0.01d	0.5fgh

Table 8. Mean comparison of characteristics for interaction between genotypes and hormonal combination in hypocotyl explant.

Genotype	Hormonal combination	Root	Airbud
Azar Gol	NAA + BAP	2.25b	0.01b
Azar Gol	NAA + KIN	4a	0.75a
Azar Gol	IAA + BAP	0.25c	0.25b
Azar Gol	IAA + KIN	0.01c	0.25b
Azar Gol	2,4-D + BAP	0.01c	0.01b
Azar Gol	2,4-D + KIN	0.01c	0.01b
Farokh	NAA + BAP	0.01c	0.01b
Farokh	NAA + KIN	0.01c	0.01b
Farokh	IAA + BAP	0.01c	0.01b
Farokh	IAA + KIN	0.01c	0.25b
Farokh	2,4-D + BAP	0.01c	0.01b
Farokh	2,4-D + KIN	0.01c	0.01b
Master	NAA + BAP	0.01c	0.01b
Master	NAA + KIN	2.5b	0.01b
Master	IAA + BAP	0.01c	0.01b
Master	IAA + KIN	0.01c	0.25b
Master	2,4-D + BAP	0.01c	0.01b
Master	2,4-D + KIN	0.01c	0.01b
CIRENA	NAA + BAP	4a	0.01b
CIRENA	NAA + KIN	0.5c	0.01b
CIRENA	IAA + BAP	0.01c	0.01b
CIRENA	IAA + KIN	0.01c	0.01b
CIRENA	2,4-D + BAP	0.01c	0.01b
CIRENA	2,4-D + KIN	0.01c	0.01b
ESBIBA	NAA + BAP	0.01c	0.01b
ESBIBA	NAA + KIN	4a	0.01b
ESBIBA	IAA + BAP	0.01c	0.01b
ESBIBA	IAA + KIN	0.25c	0.01b
ESBIBA	2,4-D + BAP	0.01c	0.01b
ESBIBA	2,4-D + KIN	0.01c	0.01b

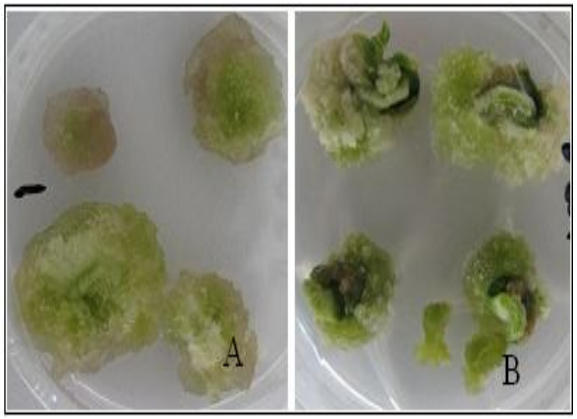

Fig. 1. A. Production of callus in cotyledon explant, Master genotype influenced by hormonal combination (NAA+ BAP).

B. Production of callus in hypocotyl explant, Master genotype influenced by hormonal combination (NAA+ KIN).

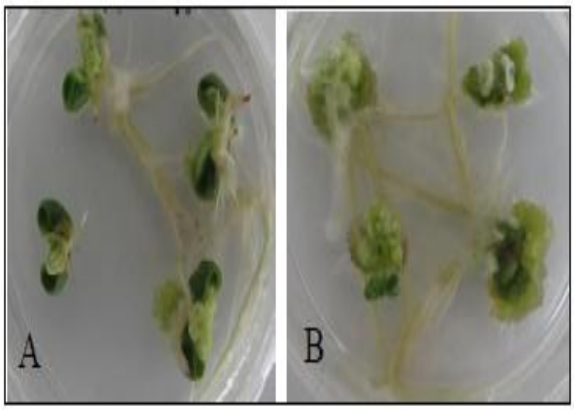

Fig. 2. A. Production of root in cotyledon explant, Azargol genotype influenced by hormonal combination (NAA+ BAP).

B. Production of root in hypocotyl explant, Azargol genotype influenced by hormonal combination (NAA+KIN).

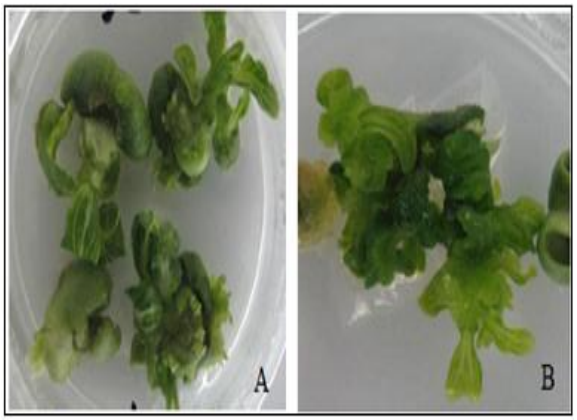

Fig. 3. A. Production of Air bud in cotyledon explant, Master genotype influenced by hormonal combination (IAA+BAP).
B. Production of Air bud in hypocotyl explant, Farokh genotype influenced by hormonal combination (IAA+KIN).

In hypocotyl explant, most mean was found in medium which was containing hormonal combination (NAA+KIN) in Azargo genotype as well as least amount was observed in media which were containing hormonal combinations (IAA+BAP) and (IAA+KIN) in Azargol genotype and medium which was containing hormonal combination (IAA+KIN) in Master and Farokh genotypes (Table 8). Grain refining will be done, when ratio of cytokinin is more than of auxin but interaction of endogenous and exogenous hormones makes the differentiation of tissues in the glass (Rout *et al.*,2006). Our results in this case were corresponded with the results of Swankar and Bohra on *P. Sumniferum* (Swankar and Bohra,1989). The results of this study showed that we can activate system of rooting, callusing and production of airbud by using the suitable explant and effective combination of hormones auxin and cytokinin. Moreover, we can obtain samples with high performance of regeneration that they have ability to become a complete plant.

References

Azadi P, Moieni A, Ahmadi MR. 2002. Shoot organogenesis from cotyledons of sunflower. Helia **25(37),** 19-26.

Baker CM, Munoz-Fernandez N, Carter CD. 1999. Improved shoot development and rooting from mature cotyledons of sunflower. Plant Cell, Tissue and Organ Culture **58,** 39-49. http://dx.doi.org/10.2298/HEL0848017F

Baskaran P, Jayabalan N. 2006. *In vitro* mass propagation and diverse callus orientation on *Sesamum indicum* L.-an important oil plant. Journal of Agricultural Technology **2(2),** 259-269.

Berrios EF, Gentzbittel L, Serieys H, Alibert G, Sarrafi A. 1999. Influence of genotype and gelling agents on *in vitro* regeneration by organogenesis in sunflower. Plant Cell, Tissue and Organ Culture **59,** 65-69.

Ceriani MF, Hopp HE, Hahne G, Escand AS. 1992. Cotyledons: an explant for routine regeneration of sunflower plants. Plant Cell Physiology **33(2),** 157-164.

Charriere F, Sotta B, Miginiac E, Hahne G. 1999. Induction of adventitious shoots or somatic embryos on *in vitro* cultured zygotic embryos of *Helianthus annuus*: variation of endogenous hormone levels. Plant Physiology Biochem **37(10),** 752-757.

Espinasse A, Lay C, Volin J. 1989. Effect of hormone concentration and explant size on shoot organogenesis from callus derived from zygotic embryos of sunflower (*Helianthus annuus* L.) Plant Cell, Tissue and Organ Culture **17,** 7-8.

Evans LS, Harangozo AM. 1982. Relationship between root morphogenesis and period of predominant cell arrest in intact an aseptically-cultured roots of *Helianthus annuss* L. Annals of Botany **49(2),** 141-147.

Fabijan D, Yeung E, Murherjee I, Reid DM. 1981. Adventitious rooting in hypocotils of sunflower (*Helianthus annuus* L.) seedlings. Physiologia Plantarum **53,** 578–588. http://dx.doi.org/10.1111/j.13993054.1994.tb08805.x

Greco B, Tanzarella OA, Carrozzo G, Blanco A. 1984. Callus induction and shoot regeneration in sunflower (*Helianthus annuus* L.). Plant Science Letter **36,** 73–77.

Hashemabadi D, Kaviani B. 2008. Rapid micro-propagation of *Aloe vera* L. via shoot multiplication. African Journal of Biotechnology **7(12),** 1899-1902.

Hassani B, Saboora A, Radjabia T, Fallah-Husseini H. 2008. Somatic Embryogenesis of *Ferula assafoetida*. JUST **33(4),** 15-23.

Ikeda M, Matsumura M, Kamada H. 2005. Suitability of small and branching sunflower varieties for molecular genetic experiments and their transformation by *Agrobacterium* infection. Plant Biotechnology **22(2),** 97-104.

Knittel N, Escand_n AS, Hahne G. 1991. Plant regeneration at high frequency from mature sunflower cotyledons. Plant Science **73,** 219-226.

Mayor ML, Nestares G, Zorzoli R, Picardi LA. 2003. Reduction of hyperhydricity in sunflower tissue culture. Plant Cell, Tissue and Organ Culture **72,** 99-103.

Murashige T, Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum **15,** 473- 497.

Nestares G, Zorzoli R, Mroginski L. 1996. Plant regeneration from cotyledons derived from mature sunflower seeds. Helia **19,** 107-112.
http://dx.doi.org/10.2298/HEL1256009C

Nestares G, Zorzoli R, Mroginski L, Picardi L. 2002. Heriability of in vitro plant regeneration capacity in sunflower. Plant Breeding **121(4),** 366-368.
http://dx.doi.org/10.2298/HEL1256009C

Ozyigit II, Bajrovic K, Gozukirmizi N, Semiz BD. 2002. Direct plant regeneration from hypocotyl and cotyledon explants of five different sunflower genotypes (*Helianthus annuus* L.) from Turkey, Biotechnology and Biotechnological Equipment **16 (1),** 8-11.

Ozyigit II, Gozukirmizi N, Semiz BD. 2006. Callus induction and plant regeneration from mature embryos of sunflower. Russian Journal of Plant Physiology **53(4),** 556-559.

Paterson KE, Everett NP. 1985. Regeneration of *Helianthus annuus* inbred plants from callus. Plant Science **42,** 125– 132.

Power CJ. 1987. Organogenesis from *Helianthus annuus* inbreds and genotypes from the cotyledons of zygotic embryos. American Journal of Botany **74,** 497– 503.
http://www.jstor.org/stable/2443828

Punia MS, Bohorova NE. 1992. Callus development and plant regeneration from different explants of six wild species of sunflower (*Helianthus annuus* L.). Plant Science **87,** 79- 83.
http://dx.doi.org/10.2298/HEL0338039P

Rout G, Mohapatra A, Mohan S. 2006. Tissue culture of ornamental pot plant: A critical review on present scenario and future prospects. Biotechnology Advances **24,**531- 560.

Sarrafi A, Bolandi AR, Berville A, Alibert G. 1996. Analysis of cotyledon culture to measure genetic variability for organogenesis parameters in sunflower (*Helianthus annuus* L.) Plant Science **121(2),** 213- 219.

Shah MI, Jabeen M, Ilahi I. 2003. *In vitro* callus induction, its proliferation and regeneration in seed explants of wheat (*Triticum aestivum* L.) variety Lu-26S. Plant Journal of Botany **35(2),** 209- 217.
http://dx.doi.org/10.5897/AJB10.770

Swankar PL, Bohra SP. 1989. Regeneration of shoots buds from callus cultures of *Papaver*

somniferum. Current Science **58,** 1382- 1384.

Sujatha M, Prabakaran AJ. 2001. High frequency embryogenesis in immature zygotic embryos of sunflower. Plant Cell, Tissue and Organ Culture **65,** 23- 29.

Velcheva M, Faltin Z, Vardi A, Eshdat Y, Peral A. 2005. Regeneration of *Aloe arborescens* via organogenesis from young inflorescences. Plant Cell, Tissue and Organ Culture **83,** 293- 301.

Vuylsteker C, leleu O, Rombour S. 1997. Influence of BAP and NAA on the expression of nitrate reductase in excised chicory roots. Journal of Experimental Botany **48,** 1079-1085. http://dx.doi.org/10.5897/AJB10.401

Zhang Y, Bhalla PL. 1999. Shoot regeneration potential from seedling explants of Australian cultivars of oil seed rape (*Brassica napus* L.). New Horizos for an old crop. Proceeding of 10th International Rapeseed Congress, Canberra, Australia.