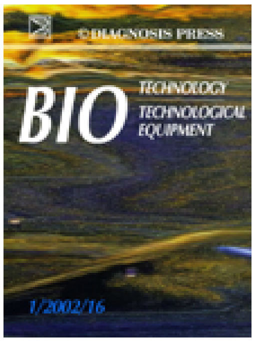

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Direct Plant Regeneration from Hypocotyl and Cotyledon Explants of Five Different Sunflower Genotypes (*Helianthus Annuus* L.) from Turkey

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Agriculture and Ecology

DIRECT PLANT REGENERATION FROM HYPOCOTYL AND COTYLEDON EXPLANTS OF FIVE DIFFERENT SUNFLOWER GENOTYPES (*Helianthus annuus* L.) FROM TURKEY

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ABSTRACT

This study aims to establish plant tissue culture and regeneration systems of five different sunflower (Helianthus annuus L.) genotypes: Trakya 259, Trakya 80, Trakya 129, Trakya 2098 and Viniimk 8931, which are commercially important for Turkey. Plant tissue culture systems were established on Murashige and Skoog (MS) media supplemented with various plant growth regulators using hypocotyl and cotyledon explants. The highest shoot regeneration was observed using hypocotyl explants with Trakya 259 genotype (40 %) on MS media supplemented with 1 mg/l BAP (6-benzylaminopurine) and 0.5 mg/l NAA (α-naphthalene acetic acid). Hypocotyl explants from other genotypes showed regeneration efficiencies as followed: Trakya 80, 33 %; Trakya 129, 29 %; Trakya 2098, 22 % and Viniimk 8931, 19 %. Shoot regeneration efficiencies with the cotyledon explants on the same medium were lower in comparation with hypocotyl explants as followed: Trakya 129, 20 %; Trakya 2098, 10 % and Viniimk 8931, 9 %. In addition, two genotypes (Trakya 259 and Trakya 80) were non-responsive on the same media with cotyledon explants. All of the regenerated shoots were rooted on MS media supplemented with 1 mg/l IBA (indol-3-butiric acid). The results obtained in this study will be useful for the improvement of gene transfer systems to these commercially important sunflower genotypes.

Introduction

Sunflower is one of the most commercially important oil crop in the world. Its seed oil can be used for human consumption, as well as a raw material for oil chemistry. It also can substitute mineral oil in various applications such as, fuels, lubricants, or oils for hydraulic systems (5). Applications of new biotechnological techniques such as gene transfers for improvement of sunflower are mainly limited by the tissue culture response of commercial varieties (6, 7, 10, 14). From first regeneration of sunflower by Sadhu (19) up to now, there are different reports on using explants such as immature embryo, shoot tip, leaf, hypocotyl, cotyledon and anther for both direct and indirect way of regeneration (1, 3, 4, 15, 16, 18).

For direct shoot regeneration of *Helianthus* cotyledons and young hypocotyls are advantageous explants, since they are easily and quickly available and posses a high efficiency (1, 2). However, shoot regeneration (by organogenesis) from other explants of sunflower remains problematic. The regeneration frequency and the number of shoots are highly variable, depending upon the genotype, the hormonal composition of the medium and the nature of the explants (9, 11).

In this study we report direct shoot regeneration of five commercially important cultivars of sunflower (*Helianthus annuus* L.) in Turkey. Four of them were originated and certificated in Turkey and the Viniimk 8931 was originated from Russia. We had found that hypocotyl explants were more capable in regeneration response in comparation with other explants.

Materials and Methods

Seeds of five commercially important genotypes of sunflower (*Helianthus annuus* L.) Trakya 259, Trakya 80, Trakya 129, Trakya 2098, Viniimk 8931 were obtained from Trakya Agricultural Research Institute, Edirne. They were surface sterilised by immersion in 70 % ethanol (3 minutes), followed by keeping in 20 % commercial sodium hypochloride (15 minutes) and rinsed 3 times (5 minutes) with sterile distilled water. Then seeds were dried onto filter papers, and cultured on MS (Murashige Skoog) free medium (12), with 30 g sucrose and 9 g agar. The pH of the media was adjusted to 5.7 with 1 M NaOH prior to autoclaving. Cultured seeds were kept at growth chamber with photoperiod of 16 hours light (3000 lux) and 8 hours dark, at 25°C and 70 % humidity.

After 10 days, hypocotyls and cotyledons of the seedlings were separated and cultured on MS media containing 1 mg/l 6-benzylaminopurine (BAP) and 0.5 mg/l α-naphthalene acetic acid (NAA) for direct regeneration. Hypocotyls were cut into 0.5 cm long pieces and cotyledons were cut transversally into two parts. Regeneration efficiencies were measured by counting the main number of shoots per explant after subcultured for 20 days intervals.

Obtained plantlets were rooted on MS without any hormone and on MS medium containing 1 mg/l indol-3-butiric acid (IBA). Rooted plants became ready to be transferred to the soil after 15 days.

Results and Discussion

The germination frequency in 5 tested cultivar seeds were around 90 %. Obtained hypocotyl and cotyledon explants were cultured on MS media supplemented with 1 mg/l BAP and 0.5 mg/l NAA. After 10 days, both hypocotyl and cotyledon explants became larger. Shoot regeneration obtained after 20 days from both hypocotyl and cotyledon explants are shown in **Table**.

TABLE

Regeneration efficiency from hypocotyl and cotyledon explants in five different sunflower genotypes on MS medium supplemented with 1 mg/l BAP and 0.5 mg/l NAA

Genotypes	Hypocotyl		
	Number of explants used	Number of shoots	Regeneration efficiency (%)
Trakya 259	20	8	40
Trakya 80	21	7	33
Trakya 129	17	5	29
Trakya 2098	18	4	22
Viniimk 8931	21	4	19
Cotyledon			
Trakya 259	25	-	0
Trakya 80	20	-	0
Trakya 129	20	4	20
Trakya 2098	20	4	10
Viniimk 8931	22	2	9

Shoot regeneration from hypocotyl explants of the tested genotypes was between 19-40 %. Trakya 259 genotype gave the best response (40 %) while Viniimk 8931 genotype showed the lowest regeneration efficiency (19 %). Regeneration efficiencies were lower using cotyledon explants. From cotyledon explants, the best response was obtained from Trakya 129 genotype

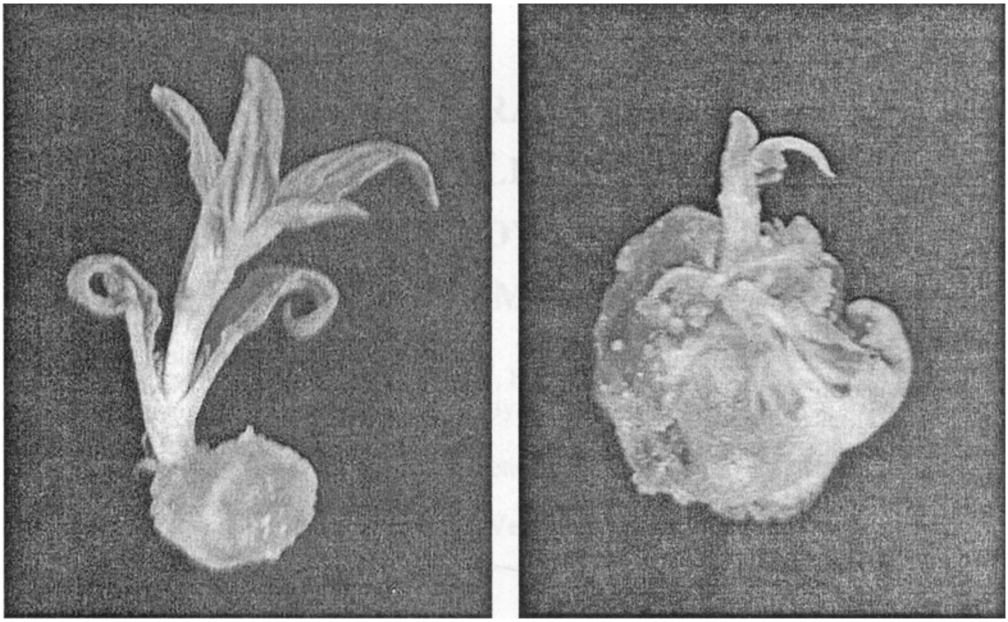

Figure. Shoot regeneration from hypocotyl (left) and cotyledon (right) explants of Trakya 259 genotype on MS media supplemented with 1 mg/l BAP and 0.5 mg/l NAA

(20 %), Trakya 2098 (10 %), and Viniimk 8931 (9 %). There was no response with the other genotypes (Table). This experiment demonstrated that regeneration efficiency depends on the genotype.

All shoots were rooted on both MS free and MS media containing 1 mg/l IBA (100 %), but MS containing 1 mg/l IBA provided well developed roots.

In our study, hypocotyl and cotyledon explants of sunflower plants (10 days old) were used for direct shoot regeneration. On MS media with 1 mg/l BAP and 0.5 mg/l NAA, Trakya 259 genotype (using hypocotyl explants) gave the highest shoot regeneration (40 %). In a similar study by Gürel (6), using hypocotyl explants, 5 different genotypes and 7 different media have been tested and shoot regeneration and callus formation was observed. MS media with 1 mg/l GA_3, 3 mg/l kinetin and 0.5 mg/l NAA were used and regeneration was 4 % for 7 day old cotyledons of Semu FV/89 Festive genotype (6,1). In addition Paterson and Everett (17) observed good regeneration and somatic embryogenesis for 12 day old hypocotyls on MS supplemented with 6.9 g/l KNO_3, 40 mg/l adenine sulphate, 500 mg/l casamino acids, 1 mg/l BAP, 1 mg/l NAA and 0.1 mg/l GA_3. Different results obtained from different studies can be explained by the difference in the plant hormones used, genotype differences and difference in explant ages.

The best condition for shoot induction of sunflower was found to occur on MS media with 0.5 mg/l NAA combined with 1 mg/l BAP (9). Using cotyledon explants (10 day old) on same medium, we found that the Trakya 129 gave the best regeneration with a percentage of 20 % but no regeneration was observed for Trakya 259 and Trakya 80 genotypes. In a similar study, Knittel et al. (9) observed the best regeneration efficiency with 4-10 day old cotyledons of 8 different genotypes on 17 different combinations of BAP and NAA. They found that using with 1mg/l BAP and 0.5 mg/l NAA gave the best result for HA300B genotype (80 %). Khalid et al. (8) used 1-4 day cotyledons of 3 different genotypes in a liquid medium with 4.4 μM BAP and 5.4 μM NAA and found that the best regeneration (59 %) belongs to 2 day cotyledons of R897 genotypes after 4 weeks. Ceriani et al (2) 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 BAP and 0.75 mg/l NAA (90 %). In a similar study, Nataraja and Ganapathi (13) used 1 week old cotyledons in 0.5 mg/l BAP, 5 mg/l IAA, 0.5 mg/l BAP, 0.5 mg/l IBA and received direct regeneration in 3-4 weeks. As it can be seen from the studies above, especially in sunflower tissue cultures, cotyledon explants that are good regeneration materials show different regenerative behaviours when kept in a culture, depending on their genotype. Age of the explant used is also an important parameter affecting the regeneration percentage.

Hypocotyls and cotyledons of young sunflower plantlets represent convenient explants for the initiation of cultures and they also have considerable potential for regenerating shoots, then quickly give rise to whole plants. The expression of this potential is influenced by a number of diverse factors. Some of them related to the plant material such as age or genotype and others to the culture conditions such as hormones, medium composition, or other physical culture conditions. This will be the first step of the biotechnological studies, especially gene transfers of these important genotypes for Turkey.

REFERENCES

1. **Belloni P.** (1992) Annals of Biology, 1397-1399.
2. **Ceriani M.F., Hopp H.E., Hahne G., Escandón A.S.** (1992) Plant Cell Physiology, **33(2)**, 157-164.
3. **Finer J.J.** (1987) Plant Cell Reports, **6**, 372-374.
4. **Fiore M.C., Trabace T., Sunseri** (1997) Plant Cell Reports, **16**, 295-298.
5. **Friedt W.** (1996) Helia, **19**, 161-179.
6. **Gürel A.** (1994) Ege Üniversitesi Ziraat Fakültesi Dergisi, **31(1)**, 41-48.
7. **Hunold R., Burrus M., Bronner R., Duret J.P., Hahne** (1995) Plant Science, **105**, 95-109.
8. **Khalid M., Chrabi B., Castelle J.C., Latche A., Roustan J.P., Fallot J**, (1992) Plant Cell Report, **10**, 617-620.
9. **Knittel N., Escandón A.S., Hahne G.** (1991) Plant Science, **73**, 219-226.
10. **Knittel N., Gruber V., Hahne G., Lénée, P.** (1994) Plant Cell Reports, **14**, 81-86.
11. **Laparra H., Stoeva P., Ivanov P., Hahne G.** (1997) Plant Cell Report, **16**, 692-695.
12. **Murashige T., Skoog F.** (1962) Physiologica Plantarum, **15**, 473-479.
13. **Natajara K., Ganapathi T.R.** (1989) Indian Journal of Botany, **27**, 777-779.
14. **Nestares G., Zorzoli R., Mroginski L., Picardi, L**, (1996) Helia, **24**, 107-112.
15. **Nurhidayah T., Horn R., Röcher T., Friedth** (1996) Plant Cell Reports, **16**, 167-173.
16. **Paterson K.E.** (1984) American Journal of Botany, **71**, 925-931.
17. **Paterson K.E., Everett N.P.** (1985) Plant Science, **42**, 125-132.
18. **Punia M.S., Bohorova N.E.** (1992) Plant Science, **87**, 79-83.
19. **Sadhu M.K.** (1974) Plant Cell Reports, **42**, 125-132.