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MORPHOGENESIS OF *in vitro* CULTURED TISSUES AND IMMATURE GERMS OF SUNFLOWER HYBRIDS (*Helianthus annuus* L.)

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SUMMARY

Cotyledons, roots, hypocotyls and immature germs of sunflower hybrids have been tested for morphogenesis. High morphogenetic ability was revealed in the cotyledon explants, hypocotyls and immature germs. Explants of hybrids Poglad, Kiy and Svitoch showed the best ability for morphogenesis. The obtained shoots were rooted for development of complete plants. The effect of „premature flowering" was discovered in all cases.

Key words: explants, hybrids, medium, morphogenesis, sunflower

INTRODUCTION

The sunflower is the major edible oil crop in the Ukraine. The biotechnology methods enable us to create new starting selection material of sunflower which can be used for obtaining new, highly productive forms.

Depending on sucrose concentration in culture medium (F. Charrière and G. Hahne, 1998), somatic embryos or shoots can be induced in *in vitro* conditions from immature zygotic embryos of sunflower (*Helianthus annuus* L.). A medium containing 3% sucrose induces only shoots while somatic embryos are formed in a medium containing 12% sucrose. Studies have been conducted on the effect of the external supply of some growth regulators known to induce organogenesis, such as cytokinins, or somatic embryogenesis, such as auxins and abscisic acid (ABA). Addition of auxins or abscisic acid to the organogenic medium (3% sucrose) induces somatic embryogenesis whereas addition of auxin polar transport inhibitors leads to shoot formation under otherwise embryogenic conditions (12% sucrose). Moreover, among the cytokinins tested, zeatin has had an unexpected embryogenic effect independently of the sugar concentration in the medium.

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Another investigation (Charrière, Sotta, Miginiac, Hahne, 1999) was conducted for better understanding of the role of endogenous hormones. The concentration of benzyladenine (BAP) was 2-fold higher in immature zygotic embryos under caulogenic conditions than in the embryogenic medium. The indolyl acetic acid (IAA) concentration peaked in the sample taken at 24 h, the time when the responding cells become determined towards one or the other morphogenic reaction. The IAA/BAP ratio was higher in the immature zygotic embryos that induced somatic embryogenesis compared with those that gave rise to shoots.

Adventitious roots of sunflower (*Helianthus annuus*) were formed quickly in plumules on MS [1/2] (Eduardo Sobrino Vesperinas, 1998). After 7 days, the plantlets could be transplanted to sterilized peat moss. The optimal IAA concentration for adventitious root formation was 0.9 mg l^{-1}, although longest roots were obtained at 0.3 mg l^{-1} IAA.

As is well known, asymmetrical division is the earliest event in embryos forming from protoplasts. Therefore general quantity of reveals on 100 protoplasts and asymmetrical divisions on 100 protoplasts in 52 recombinant inbred lines from crosses of sunflower lines PAC-2 and RHA-266 in protoplast culture were investigated (Flores Berrios *et al.*, 2000). It was revealed that 12 loci were associated with general quantity of reveals on 100 protoplasts and 11 loci with asymmetrical divisions on 100 protoplasts.

Statistical analysis of F_1 hybrids from crosses between three cytoplasmic male sterile and six restorer sunflower inbred lines and their parents showed a high genetic variability for organogenetic parameters (Sarrafi *et al.*, 1996). Both general and specific combining abilities were significant for all organogenetic parameters under study.

MATERIAL AND METHODS

The hybrids Harkov 49, Svitoch, Kiy and Poglad were the objects of our investigation. Their seedlings, roots, hypocotyls and immature germs were tested for morphogenesis.

The seeds were surface sterilized in water (30 min.), 96% ethanol non-hull (1 min.), 96% ethanol hull (1 min.), 2.5% NaOCl (20 min.) and rinsed three times in distilled water (15 min.). The sterile sunflower plants were grown in water agar and used as explants of somatic tissues.

Immature embryos of hybrids Kharkov 49, Svitoch, Kiy and Poglad were obtained from open-pollinated greenhouse-grown plants. Immature seed heads were cut when the pericarps had acquired a dark color, two weeks after pollination, but before moisture content began to decrease. The seeds were surface sterilized as described in the previous paragraph.

We used MS–medium (T. Murashige and F. Skoog, 1962). Regeneration capacity was tested with different concentrations of sucrose, BAP, NAA, 2,4-D:

1. MS+BAP-4mg/l, NAA-0.5 mg/l, sucrose -30 g/l,
2. MS+BAP-12mg/l, NAA-0.1 mg/l, sucrose -30 g/l,
3. MS+BAP-1 mg/l, sucrose -60 g/l,
4. MS+2,4-D-10mg/l, NAA-0.5 mg/l, BAP-1.5 mg/l, sucrose -30 g/l,
5. MS+BAP-1mg/l, NAA-0.5 mg/l, sucrose -30 g/l.

Root induction medium consisted of basic MS macro and micro salts, glutamine (200 mg/l), sucrose (30 g/l), agar (8 g/l) (Witrzens, Scowcroft, Downes, Larkin, 1988).

The explants were incubated at 20°C under light (1000 lux) and photoperiod 16/8 h.

RESULTS

All of the tested hybrid explants showed callus induction ability. The best caulogenic ability was indicated in the hybrid Poglad. Explants exhibited most intensive callus formation in medium 1. Hypocotyls of the hybrids Svitoch and Kiy formed compact calli with embryogenetic zones, on which multiple shoots developed after 4 weeks.

Figure 1: Multiple shoots formation from cotyledon segments grown in vitro.

In medium 2, the majority of explants of all hybrids developed watery calli in small amounts. In this medium only immature germs of the hybrid Svitoch formed a large volume of light calli on which occurrence of embryonic structures with small leaves was observed after 4 weeks of cultivation.

In medium 3, the explants formed insignificant amounts of dense greenish calli. Hypocotyls of the hybrid Poglad formed 3-4 of adventitious shoot structures of dense calli after 4 weeks of cultivation. The formation of numerous small leaves and shoot structures was observed on immature germs of the same hybrid.

In the case of medium 4, all genotypes formed few light calli, which later on turned brown and perished after 5 weeks of cultivation.

The light dense calli formed in medium 5. On seedling explants of the hybrid Poglad, direct regeneration with formation of 3 to 6 shoot structures (up to 1 cm) was observed. The regeneration with formation of many small leaves and shoot structures was also observed in the case of immature germs of the hybrid Svitoch.

The obtained regenerants were transplanted onto root induction medium. Flowering of the majority of regenerants and occurrence of well advanced roots was observed. The phenomenon of „premature flowering" is not undesirable for obtaining functional seeds in the progeny.

CONCLUSIONS

Thus, high morphogenic ability was revealed for seedling explants, hypocotyls and immature germs. Explants of isolated roots of the investigated genotypes formed only insignificant amounts of light calli in all mediums. The best mediums for direct regeneration were MS+BAP-1 mg/l, sucrose-60 g/l and MS+BAP-1 mg/l, NAA-0.5 mg/l, sucrose -30 g/l.

The medium which contained 2,4-D depressed callus growth. The medium MS+BAP-12 mg/l, NAA-0.1 mg/l, sucrose -30 g/l was best for embryogenesis from immature germs. Explants of the hybrids Poglad and Svitoch showed the best morphogenetic ability.

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MORFOGÉNESIS *in vitro* DEL TEJIDO CULTIVADO Y DE LOS GÉRMENES INMADUROS EN LOS HÍBRIDOS DE GIRASOL (*Helianthus annuus* L.)

RESUMEN

Fue investigada la morfogénesis de cotiledones, raíz, hipocótilos y los gérmenes inmaduros en los híbridos de girasol. Se ha determinado una alta capacidad morfogenética en los explantos de cotiledones, hipocótilos y los gérmenes inmaduros. Los explantos de los híbridos Poglad, Kiy y Svitoch han mostrado la más alta capacidad de morfogénesis. Los brotes obtenidos son arraigados, para que se obtengan las plantas completas. La influencia de "floración precoz" fue determinada en todos los casos.

MORPHOGENÈSE DE TISSUS CULTIVÉS *in vitro* ET DE GERMES IMMATURES D'HYBRIDES DE TOURNESOL (*Helianthus annuus* L.)

RÉSUMÉ

La morphogenèse des cotylédons, racines, hypocotyles et germes immatures d'hybrides de tournesol a été étudiée. Une grande aptitude morphogénétique a été révélée dans les cotylédons des explants, les hypocotyles et les germes immatures. Les explants des hybrides Poglad, Kiy et Svitoch ont montré la meilleure aptitude de morphogenèse. Les pousses obtenues ont été enracinées pour permettre le développement de plantes complètes. L'effet «floraison prématurée» a été constaté dans tous les cas.