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SOMATIC EMBRYOGENESIS AND ORGANOGENESIS INDUCED IN IMMATURE ZYGOTIC EMBRYOS OF SELECTED SUNFLOWER (*HELIANTHUS ANNUUS* L.) GENOTYPES

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In vitro somatic embryogenesis and organogenesis (shoot and root formation) were observed when immature zygotic embryos of the sunflower cultivars Frankasol, Printasol, Lech and Wielkopolski were cultured on JME medium containing high sucrose concentration (350 mM). Of four tested cultivars, Frankasol showed the highest rate of somatic embryogenesis, and Printasol the highest rate of organogenesis. The remaining two cultivars responded slightly.

Key words: *Helianthus annuus* L., sunflower, zygotic embryos, regeneration, somatic embryogenesis, organogenesis.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the world's major oil seed crops and the object of intense breeding programs. Several reports have described methods to regenerate cultured sunflower tissues from different explants such as immature zygotic embryos (Finer, 1987; Freyssinet and Freyssinet, 1988, Jeannin et al., 1995) and mature cotyledons (Knittel et al., 1991; Fiore et al., 1997); callus, (Espinasse and Lay, 1989; Zezul et al., 1995), and protoplasts (Binding et al., 1981). Among these explants, immature zygotic embryos (IZEs) are most frequently used because of their high morphogenic potential. Regeneration from IZEs can occur either by organogenesis or by somatic embryogenesis (Bronner et al., 1994) and for both pathways it may be either direct or indirect. The growth regulator needed in the culture medium is benzyloaminopurine (BAP) at a concentration (6.6 μM) identical for both somatic embryogenesis and organogenesis (Charriere and Hahne, 1998). The morphogenic response has been found to be influenced only by the sugar concentration (Jeannin et al., 1995). Under identical culture conditions, organogenesis is induced at low sucrose concentration (87 mM), while somatic embryogenesis occurs at high sucrose concentration (350 mM). Although IZEs are the best tissue type, which can break the regeneration barrier most successfully, plant regeneration is genotype-dependent.

The aim of our experiment was to determine the influence of sunflower genotype on somatic embryogenesis and organogenesis induction. The morphogenetic capacity to produce somatic embryos and adventitious shoots was evaluated in immature zygotic embryos of four selected genotypes. Histological analysis of the morphogenetic phenomena will be the subject of a separate paper in preparation.

MATERIALS AND METHODS

The experiments employed four commercial cultivars of *Helianthus annuus* L.: Frankasol (FR) and Printasol (PR) kindly provided by Semens Cargill (Warsaw, Poland), and Lech (LECH) and Wielkopolski (WLK) kindly provided by IHAR (Radzików, Poland).

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TABLE 1. Results of regeneration experiments in four sunflower cultivars

Genotype	Mean no. of somatic embryos/explant	Mean no. of shoots/explant	% of explants with somatic embryos	% of explants with shoots and roots
Frankasol	12.04	2.80	72.0	32.0
Printasol	2.40	2.36	52.0	60.0
Wielkopolski	0.04	0.64	4.0	28.0
Lech	0.12	0.76	8.0	12.0

Influence of genotype on somatic embryogenesis statistically significant at $p \leq 0.00$ (Kruskal-Wallis test)
Influence of genotype on shoot and root formation statistically significant at $p < 0.01$ (Kruskal-Wallis test)

The plants were grown outdoors in our department's experimental field in Modlnica near Cracow. To avoid random pollination the developing inflorescences were covered with smooth tracing paper bags. Mature flowers were pollinated by hand and immature embryos were collected 4–21 days later. Developing seeds were surface-disinfected with 70% ethanol for 1 min followed by immersion in commercial bleach (ACE – 4% sodium hypochlorite) for 30–40 min, and subsequently washed three times with sterile deionized distilled water.

Isolated immature embryos at late torpedo-shaped stage (3–4 mm in length) were taken for explant excision (Fig. 1). They were cut lengthwise, producing explants containing one half of each cotyledon and one half of the embryo axis. The wound surface of the explant was placed in contact with the medium.

The medium designated JME was used to induce morphogenic response. It consisted of MS salts (Murashige and Skoog, 1962), vitamin B_5 (Gamborg et al., 1968), 1 g/L casein hydrolysate (Fluka BioChemica), 100 mg/L myo-inositol, 500 mg/L 2[N-morpholino]ethanesulfonic acid (MES), 6.6 μM BAP (6-benzyladenine) and 0.8% (w/v) agar (Difco Bacto-Agar), and was supplemented with 12% (w/v) 350 mM sucrose (Jeannin et al., 1995). The pH was adjusted to 5.8 with NaOH before autoclaving. All cultures were kept at 25°C for the first two weeks in darkness and then in light (60 μM $m^{-2}s^{-1}$ light intensity) under a 16 h photoperiod.

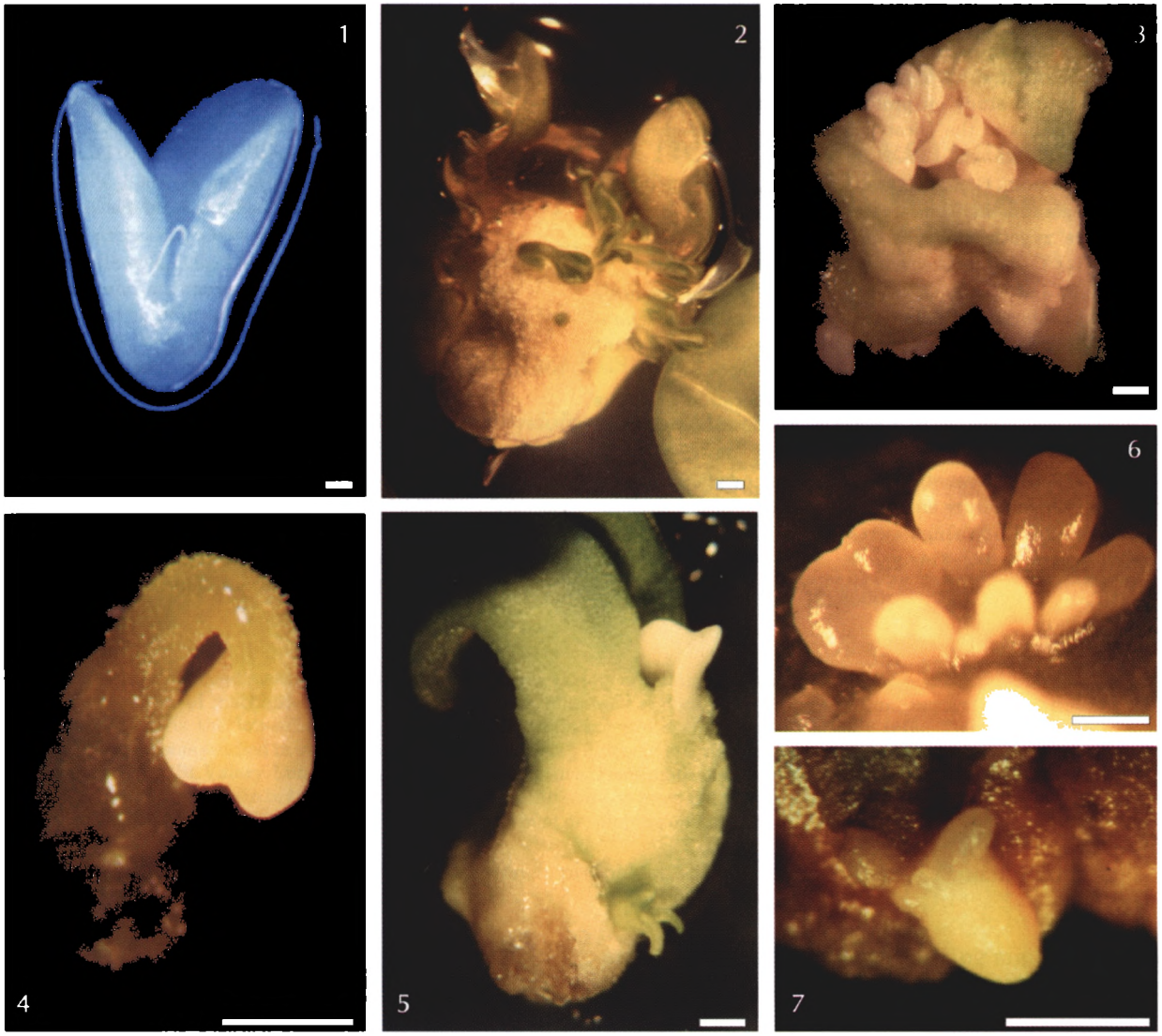
The morphogenic response was recorded after 14–20 days of culture, when somatic embryos, shoots and roots were unambiguously identifiable. The organs produced were counted under a stereomicroscope. Each experiment was repeated 5 times, with the number of explants between 25 and 30. For statistical evaluation the Kruskal-Wallis (nonparametric statistics) test was used.

Microphotographs were taken under a Zeiss Stemi SV stereomicroscope equipped with an MC 80 microphotographic attachment on Kodak film.

RESULTS AND DISCUSSION

The first signs of morphogenesis were observed on the 8th day of culture in darkness, and to the full extent after transfer to light. The morphogenic response appeared as organogenesis (shoot and root formation) or somatic embryogenesis (Figs. 2–7). The number of explants responding varied according to the genotype. The FR genotype produced the highest number of somatic embryos from explants (72%), while the PR genotype yielded the most explants showing organogenesis (60%) (Tab. 1). Generally the capacity for organogenesis in the tested cultivars was higher than the capacity for somatic embryogenesis (Tab. 1). Only in the FR cultivar did more explants produce somatic embryos, and the mean number of embryos per FR explant was significantly higher than the number of shoots and roots formed (Tab. 1). The differences in morphogenetic response were nonsignificant between cultivars WLK and LECH, but highly significant between FR and WLK and LECH.

Irrespective of the genotype, somatic embryos appeared only on the cotyledons and hypocotyls, whereas shoots were also produced from apical shoot meristem. Nevertheless, morphogenesis was observed mainly on the hypocotyl part of the explant. Shoots were never interspersed with somatic embryos (Fig. 5). Although the capacity for somatic embryogenesis and organogenesis induction differed between genotypes, darkness treatment and high sucrose concentration were necessary to obtain somatic embryogenesis in all tested cultivars.



Figs. 1–3. *Helianthus annuus* L. cv. Frankasol. **Fig. 1**. Isolated zygotic embryo at late torpedo-shaped stage. **Fig. 2.** Adventitious shoots on immature zygotic embryo. **Fig. 3.** Somatic embryos appearing directly on immature zygotic embryo. **Fig. 4.** *Helianthus annuus* L. cv. Lech. Somatic embryo excised from explant. **Fig. 5.** *Helianthus annuus* L. cv. Printasol. Adventitious shoots and somatic embryos on the same explant. **Figs. 6–7.** *Helianthus annuus* L. cv. Frankasol. **Fig. 6.** Somatic embryogenesis preceded by callus formation. **Fig. 7.** Single somatic embryo. Bars in Figs. 1, 2, 3, 5 = 1 mm; in Figs. 4, 6, 7 = 0.5 mm.

In general, adventitious shoots and somatic embryos arose directly from explant tissue without callus formation. Callus formation preceded somatic embryogenesis in only a few cases in Frankasol and Lech (Fig. 6).

This study shows that somatic embryogenesis and organogenesis can be induced successfully in immature zygotic embryos of the four tested sunflower genotypes. However, the efficiency of morphogenic activity observed in late torpedo-shaped stage zygotic embryos depends on the genotype. It is highest in Frankasol for somatic embryogenesis, and in Printasol for organogenesis. Moreover, that dependence is decidedly more visible in the case of somatic embryogenesis than in organogenesis induction. The choice of genotype seems decisive in planning efficient procedures for somatic embryogenesis in sunflower.

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