

## BRIEF COMMUNICATION

**Different regeneration potential of various sunflower (*Helianthus annuus* L.) genotypes in meristem culture**

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**Abstract**

Eleven genotypes of sunflower were used to screen their regeneration potential in a meristem regeneration test. The inbred line HNK-81 showed the best regeneration of shoots directly from apical meristems and meristem derived calli, and rarely also the formation of adventitious shoots from leaves of regenerated shoots.

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Meristem cultures have been used for micropropagation of sunflower plants (Trifi *et al.* 1981, Jegla and Sussex 1987 and Lupi *et al.* 1987), but also for evaluation of the regeneration potential of various genotypes *in vitro* (Bohorova *et al.* 1985, Paterson and Everett 1985, Robinson *et al.* 1987).

Successful initiation of tissue cultures and subsequent regeneration of plants are often dependent on the selection of suitable genotypes (Keyes *et al.* 1980 and Dietert *et al.* 1982).

Sunflower seeds were obtained from Krasnodar Breeding Station, Ukraine (cultivars Voronezhskii and Jenisei) and State Examination Station Bratislava, Slovak Republic (inbred lines GK-70, NS-H-41, NSA-26, Davil, Emil, Cammel, Francasol, Lotus and HNK-81).

All seed material was sterilized for 30 s in 70 % ethanol and 15 min in 10 % calcium hypochlorite solution, and then rinsed four times with sterile distilled water. Sterile seeds were germinated on an agar-solidified MS medium (Murashige and Skoog 1962) without growth regulators, and maintained for 14 d at photoperiod 16/8 h, irradiance 11.2  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (400-700 nm) and temperature 23 - 25°C.

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Abbreviations: BAP - 6-benzylaminopurine, KIN - kinetin; MS - Murashige-Skoog medium; NAA - 1-naphthaleneacetic acid

Half-shoot apical meristems were isolated using forceps and plated on agar-solidified MS medium containing  $1.5 \text{ mg l}^{-1}$  KIN or  $2 \text{ mg l}^{-1}$  BAP having a multiplication effect (Paterson and Everett 1985) on shoot production (Fig. 2A).

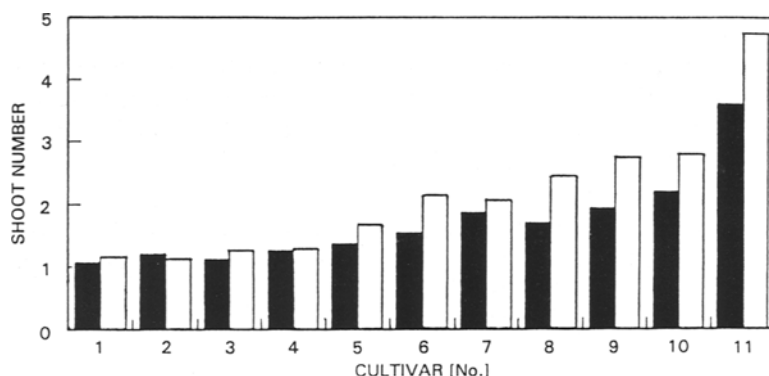


Fig. 1. Effect of sunflower genotype on the shoot multiplication and regeneration potential in meristem culture. Data obtained from 3 independent experiments showed marked variability between 11 genotypes (90 meristems in total from each genotype were tested). 1 - NS-H-41, 2 - Lotus, 3 - GK-70, 4 - Davil, 5 - NSA-26, 6 - Voronezhskii, 7 - Cammel, 8 - Francasol, 9 - Jenisei, 10 - Emil, 11 - HNK-81.

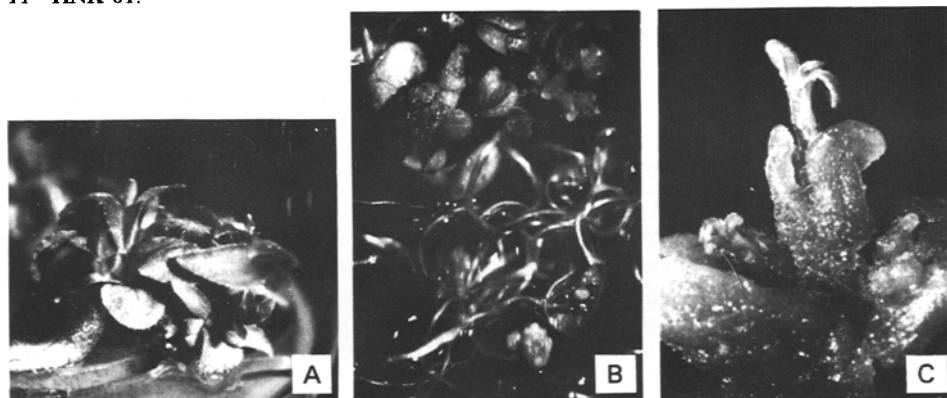


Fig. 2. A. Shoot multiplication from shoot half-apical meristem of inbred line HNK-81 on MS medium with  $2 \text{ mg l}^{-1}$  BAP. B. Rooting of regeneration shoots on MS medium with  $0.1 \text{ mg l}^{-1}$  NAA. C. Shoot organogenesis from meristem derived callus of inbred line HNK-81 on MS medium with  $4 \text{ mg l}^{-1}$  BAP.

A screening test of eleven genotypes of sunflower for shoot multiplication from a half apical meristem was made to identify the genotype with highest regeneration potential (Fig.1). Shoot production was scored by assessing the average number of shoots produced from each half-shoot apex in three independent experiments.

Regenerated shoots were rooted successfully on MS medium with  $0.05 - 0.2 \text{ mg l}^{-1}$  NAA (Fig. 2B) and were transferred to soil. Calli were induced from meristems on MS medium with  $0.2 \text{ mg l}^{-1}$  NAA and  $0.2 \text{ mg l}^{-1}$  BAP.

Inbred line HNK-81 showed the highest regeneration potential (greatest multiplication of shoots) in meristem culture, but also at the callus culture level. Organogenic callus and shoot organogenesis were rarely induced from callus (maximal 10 % frequency) of this genotype, only on MS medium with 4 mg l<sup>-1</sup> BAP (Fig. 2C).

Paterson and Everett (1985) described adventitious shoot production on leaves and petioles of regenerated shoots as phenomena closely related to regeneration potential *in vitro*. However, only the inbred line HNK-81 was able to produce adventitious shoots, but not regularly (in spite of identical, strictly controlled conditions in our experiments).

Whether such direct regeneration is a reliable indicator of the ability to regenerate plants also from callus or protoplasts has to be tested rigorously in subsequent experiments.

## References

- Bohorova, N.E., Atanasov, A., Georgieva-Todorova, J.: *In vitro* organogenesis and embryo culture in the genus *Helianthus* L. - Z. Pflanzenzücht. **95**: 35-44, 1985.
- Dietert, M.F., Barron, S.A., Yoder, O.C.: Effect of genotype on *in vitro* culture in the genus *Brassica*. - Plant Sci. Lett. **26**: 233-240, 1982.
- Jegla, D.E., Sussex, M.: Developmental potential of the sunflower (*Helianthus annuus* L.) terminal shoot meristem. - Amer. J. Bot. **74**: 616-618, 1987.
- Keyes, G.J., Collins, B., Taylor, N.L.: Genetic variation in tissue cultures of red clover. - Theor. appl. Genet. **58**: 265-271, 1980.
- Lupi, M.C., Bennici, A., Locci, F., Gennai, D.: Plantlet formation from callus and shoot-tip culture of *Helianthus annuus* L. - Plant Cell Tissue Organ Cult. **11**: 47-55, 1987.
- Murashige, T., Skoog, F.: A revised medium for rapid growth and bioassays with tobacco tissue cultures. - Physiol. Plant. **15**: 473-497, 1962.
- Paterson, K.E., Everett, N.P.: Regeneration of *Helianthus annuus* inbred plants from callus. - Plant Sci. **42**: 125-132, 1985.
- Robinson, K.E.P., Adams, D.O.: The role of ethylene in the regeneration of *Helianthus annuus* (sunflower) plants from callus. - Physiol. Plant. **71**: 151-156, 1987.
- Trifi, M., Mezghani, S., Merrakchi, M.: Multiplication végétative du Tournesol *Helianthus annuus* L. par culture *in vitro*. - Physiol. vég. **19**: 99-102, 1981.

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