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PROPAGATION AND MULTIPLICATION OF SUNFLOWER LINES (*Helianthus annuus L.*) BY TISSUE CULTURE *IN VITRO*

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SUMMARY

Callus formation could be induced from segments of different organs prepared from mature seeds or germinated seedlings of sunflower. In the latter case, callus formation was pronounced from hypocotyl and cotyledon segments, however, no plants could be regenerated from such calluses. On the contrary, multiple shoot formation was achieved from mature embryo segments and callus induced on these fragments. Genotypic differences were obvious for all types of response. Shoots could be rooted and induced to develop complete plants which could be grown to maturity. As a major obstacle, excessive "premature flowering" must be avoided in order to improve the efficiency of vegetative propagation *in vitro*.

INTRODUCTION

Cell and tissue culture techniques have been demonstrated to be useful tools for specific purposes in the course of a plant breeding programme (cf. Dunwell, 1986), where particular progress has been made in the genus *Brassica* (e.g. Siebel and Pauls, 1989).

Also in sunflower, specific techniques - like embryo culture - have been very successfully used, e.g., for rescue of interspecific hybrid embryos (Chandler and Beard, 1983; Espinasse et al., 1985; Kräuter et al., 1991). Other techniques, like anther culture, have also been applied for producing androgenetic haploids (Alissa et al., 1985; Bohorova et al., 1985; Mix, 1985; Mezzarobba and Jonard, 1986). However, this technique still needs substantial improvement since response is strongly affected by genotype, media and culture conditions (Gürel et al., 1991a). Furthermore, application of isolated microspore culture remains an unsolved problem, since not a single plant has been regenerated from such cells up till now (cf. Gürel et al., 1991b) However, improvements of these techniques should be feasible by further adjustment of donor plant cultivation, identification of the appropriate anther stage as well as media composition and culture conditions.

For such adjustments, respective experiments with responsive tissue, e.g., from the cotyle dons or the hypocotyl, can be helpful. Furthermore, successful regeneration of intact plants from such tissue is a beneficial tool for propagation and multiplication of valuable sunflower materials, e.g., male sterile female lines. In addition, tissue culture can possibly open new ways of *in vitro* selection for disease resistance (cf. Hartmann et al., 1988) and of genetic transformation of sunflower.

MATERIAL AND METHODS

The following sunflower (*Helianthus annuus L.*) lines were used for the experiments: 'Baso' (cms), 'Noba' (cms), 'HA89' (cms), 'female #499' (cms), 'female #500' (cms), and 'R429' (restorer line).

Seeds were disinfected for 10 min in a sodium hypochloride solution (2%) with a drop of detergent added and then rinsed in distilled water twice. The seeds were subsequently germinated in an incubator at 25°C and 3,000 lux light intensity on a modified MS-medium (Murashige and Skoog, 1962; cf. Kräuter, 1990). This medium was supplemented with activated charcoal (5 g/l); pH value was 5.4-5.6 (**MS2-medium**). After one week, seedlings were cut into pieces and fragments placed on another modified MS-medium (**04-medium**) with the following additions (mg/l): thiamin (0.4), meso-inositole (100), NAA (2), BAP (1), sucrose (60,000), agar agar (7-7,500); pH-value 5.4-5.6. The following variants of explants were used: intact cotyledons, hypocotyl segments (approx. 1 cm), root segments (approx. 1 cm), and shoot tips. The latter were cultured on a third, modified MS-medium for rooting (**MS1-medium**), i.e., without hormones and with reduced sucrose content (10 g/l) and solidified by agar agar (9 g/l). For each variant, 18 explants were used (6 explants in each of 3 petri disches, 5 cm). Segments and shoot tips were cultured in an incubator at 25°C under permanent light (approx. 3,000 lux). Response was recorded 2 and 6 weeks after culture initiation.

From the same sunflower genotypes, embryos were prepared from mature seeds treated in the same way as above. Of each line, 10 embryos were cut into segments (cotyledons, hypocotyl and roots) and cultured on 04-medium described above. Results were recorded 14 and 38 days later and newly formed shoots were transfered to the MS1-medium (cf. Kräuter, 1990).

RESULTS

All of the organ segments cultured on 04-medium developed calluses. The strongest reaction was obtained from hypocotyl pieces placed vertically in the agar medium. Cotyledon segments were also highly reactive, while intact cotyledons often showed hypertrophy of the tissue. The poorest reaction was observed with root segments; they only developed watery callus which later turned brown.

Sunflower inbred line 'HA89' exhibited the strongest callus formation. However, genotypic differences are biased due to partially poor seed quality. Not a single callus could be induced to regenerate plants. Only hypocotyl callus of line 'Baso' developed shoot initials. Shoot tips cultured on rooting medium (MS1-medium) showed direct shoot formation with poor root development. These shoots were transfered to soil after treatment with 'Wurzelfix' which resulted in further normal plant growth.

In the case of embryo segments, callus induction was observed in all genotypes already 3-4 days after initiation of cotyledon culture, where line 'Noba' showed particularly strong reactivity. Multiple shoot formation was already present 2 weeks after beginning of culture in all of the lines (Fig. 1a,b). This development occurred on the initation medium without transfer onto a specific regeneration medium.

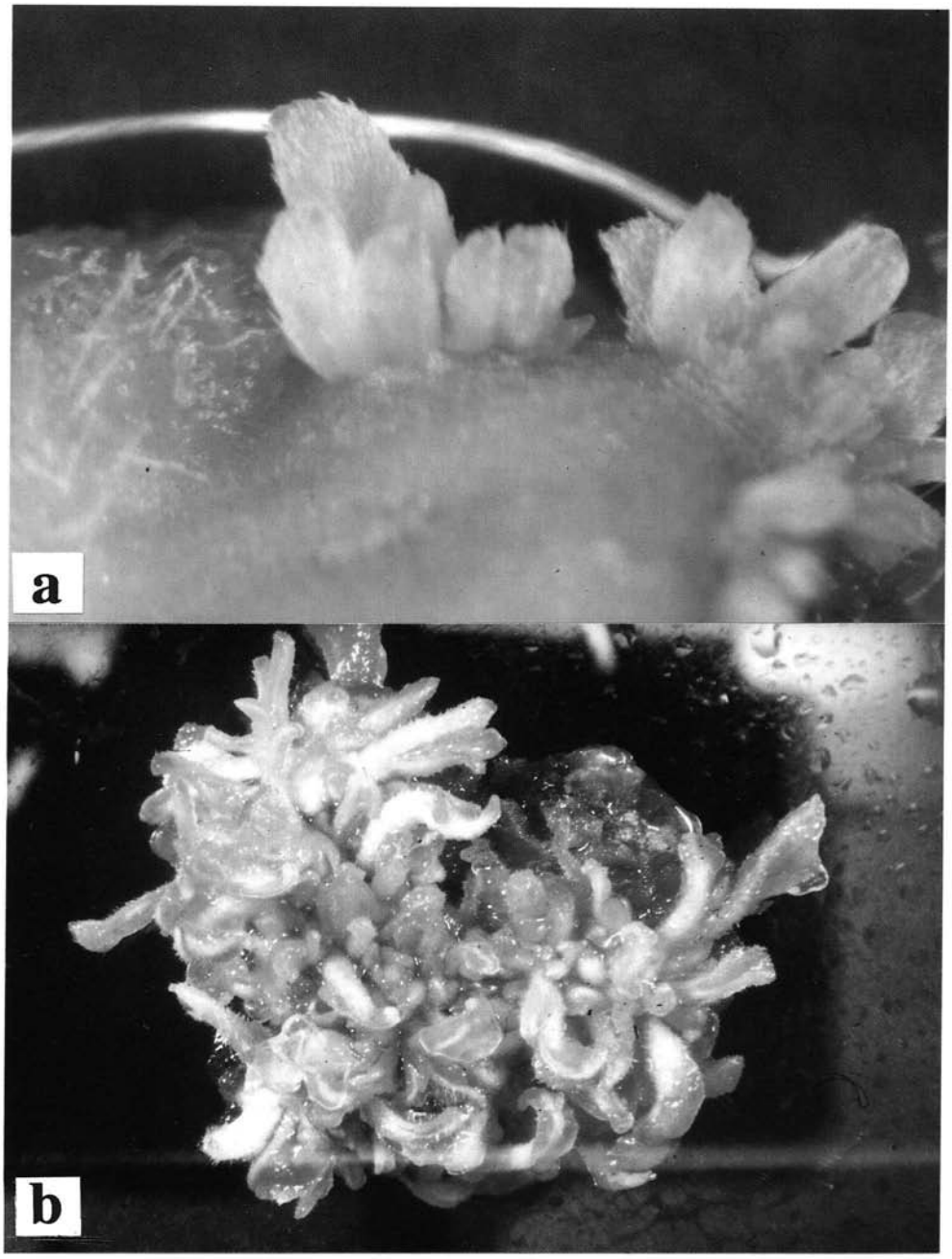

Fig.1. Multiple shoot formation from cotyledon segments cultured *in vitro*. (a) initial stage, (b) advanced stage with numerous shoots. R. Kräuter and W. Friedt

Similar response was achieved from hypocotyl and root segments of the embryo. However, differential genotypic reactions were observed in these cases, where only 'Baso', 'female #400' and 'R429' formed shoots and the other lines showed excessive callusing and root formation.

Shoots were transfered to rooting medium (MS1-medium), where plants could be finally recovered from the genotypes 'Baso', 'Noba', 'HA89', and 'female#500'. After 4 weeks, complete plants were transfered to soil in the greenhouse.

DISCUSION

Similar results to those reported here have been obtained by other authors. For example, Greco et al. (1984) and Paterson (1984) reported on multiple shoot formation from apical meristems of sunflower, whereas Power (1987) achieved direct shooting from cuttings of immature embryos. Opposite to the latter author, we received a higher shooting efficiency with higher hormone concentrations (mg/l): 2.0 vs 0.1 NAA, 1.0 vs 0.5 BAP. Similar to others reports (e.g., Paterson and Everett, 1985) our results were also clearly dependent on genotype.

In agreement with the above authors, the ability for organogenesis is drastically reduced when differentiated tissues - like segmented seedlings in our case - are cultured. Regeneration capacity is therefore maximum in juvenile, multifunctional tissue.

Unfortunately, many shoots stared to flower already *in vitro*. This phenomenon is highly undesirable and does not allow the recovery of functional seeds from such progeny. This problem, which has been reported frequently (e.g., Greco et al., 1984; Paterson, 1984), must be solved in order to improve the final success of propagation and multiplication of sunflower genotypes *in vitro*.

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PROPAGATION ET MULTIPLICATION DES LIGNÉES DE TOURNESOL (*Helianthus annuus* L.) PAR LA CULTURE DE TISSUS *in vitro*

RÉSUMÉ

La formation de cals peut être induite à partir de segments de divers organes prélevés sur des graines matures ou des germinationsde tournesol. Dans ce dernier cas, l'obtention de cals a été réalisée à partir de fragments d'hypocotyles et de cotyledons, cependant aucune plante n'a pu être régénérée à partir de ces cals induits sur ces fragments. Des différentes génotypiques étaient notables pour les diifférens types de réponses. Les pousses ont pu être enracinées et des plantes entières développées jusqu'à maturité. L'obstacle majeur réside en l'apparirion exessive de floraison prématurée, ce qui doit être évité pour améliorer l'efficacité de la propagation végétative *in vitro*.

PROPAGACION Y MULTIPLICACION DE LINEAS DE GIRASOL (*Helianthus annuus* L.) POR CULTIVO in vitro DE TEJIDO

RESUMEN

La formación de callo puede ser inducida a partir de trozos de diferentes órganos preparados a partir de semillas maduras o plántulas en germinación de girasol. En el segundo caso, la formación de callo fue pronunciada al utilizar trozos de hipocotilo y cotiledones, sin embargo no pudieron ser regeneradas plantas de tales callos. Por el contrario, la formación múltiple de callo se alcanzó a partir de trozos de embriones maduros y callo inducido sobre ellos. Las diferencias genotipicas fueron obvias para todos los tipos de respuesta. Los tallos pudieron ser enraizados e inducidos a plantas completas las cuales pudieron ser crecidas hasta madurez. Como obstáculo mayor, una floración excesivamente prematura debe ser evitado para mejorar la eficiencia de la propagación vegetativa "in vitro".