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REGENERATION POTENTIAL OF SUNFLOWER HYBRIDS

Nedev T.[1*], Vassilevska-Ivanova R.[2], Tzekova Z.[2]

[1]*Dept. Cell Genetics, Institute of Genetics, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria*
[2]*Dept. Cytogenetics, Institute of Genetics, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria*

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SUMMARY

Plant regeneration potential of two F_1 sunflower interspecific hybrids was evaluated. Immature embryos (8-10 days old) were harvested. Basal *MS* medium supplemented with 2 mg/ml zeatin proved to be better than other variants tested for callus initiation and regeneration. Regenerable calli were obtained from both hybrids. Complete plantlets were formed within 20-30 days. Part of the regenerated plants showed no major alterations in morphology as compared with the original ones.

Key words: Embryogenic callus, immature embryos, regeneration, sunflower.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is grown on more than 9.8 million hectares in the world. The introduction of new germplasm with valuable genes could play a major role in the improvement of the cultivated species. Hybrid seeds often abort because the endosperm fails to develop normally. The isolation of immature hybrid embryos by culture *in vitro* can overcome these difficulties (Gavish *et al.*, 1992).

In the past decade, rescue of hybrid sunflower embryos has been used for production of interspecific hybrids (Chandler and Beard, 1980). Production of hybrids *in vitro* depends on both the genetics of parents and the possibility for optimization of experimental conditions (age of embryos, media composition, hormone treatment, etc.) Although numerous sunflower hybrids have been regenerated from embryo rescue, further studies have to be carried out in order to improve the methodology (Chandler and Bear, 1983).

Our objective was to screen F_1 sunflower hybrids from the Institute of Genetics, Bulgarian Academy of Sciences, Sofia, for their possibility of dedifferentiation and

* Corresponding author

differentiation from immature zygotic embryos. This paper summarizes the results of the evaluation of two F_1 sunflower hybrids on four induction media (*K*, *Z1*, *Z2* and *Z3*).

MATERIALS AND METHODS

Three lines of cultivated sunflower, 1607, 2607 and 1234 (cytoplasmic male sterile), (2n=2x=34) were used as female parents. Wild *Helianthus annuus* E045, (2n=2x=34) and *Verbesina helianthoides* Michaux, (2n=2x=34) were used as pollinators. The pollinator seeds were kindly provided by the Institute of Wheat and Sunflower "Dobroudja", G. Toshevo, Bulgaria. Self-pollinated parent plants were grown in the field at the Institute of Genetics in 1995 and 1996.

Immature embryos were collected 8-10 days after pollination. Whole seads were surface-sterilized for 20 min. with 0.1% $HgCl_2$ or 20% of commercial blench both containing a few drops of Tween-80, and rinsed 3 times with sterile distilled water. Immature embryos were aseptically sliced and placed on four different variants of *MS* media (Table 1). Ten immature embryos harvested from three to five plants of each hybrid combination were tested. Cultures were maintained at 16/8 h day/night photoperiod at 23-25°C.

Table 1: Media composition for callus initiation and morphogenesis

Medium designation*	Basic macro- & micro-salt	Act. char-coal (mg/i)	Supplement (mg/l)					
			NAA	Kinetin	Zeatin	Sucrose	Vitamins	GA_3
Z1	MS	-	0.5	-	1.0	5 000	MS	-
Z2	MS	-	0.5	-	2.0	5 000	MS	-
Z3	MS	-	0.5	-	3.0	5 000	MS	-
K	MS	-	0.5	5.0	-	5 000	MS	-
P	1/2 MS	5 000	0.5	-	-	20 000	MS	0.5

*Z - zeatin, *K* - kinetin, *P* -Power C.J., *MS* - Murashige et Skoog, GA_3 - giberrelic acid

The pH of all media was adjusted to 5.8 with 0.1 N NaOH or 1N HCl and 0.7 % agar was added for solidification.

Calli or tissues obtained were transferred to a medium with 30 g/l sucrose or to the same medium. The development of embryos was evaluated after 10 days. Cultures were subcultured every 21 days. Three week old shootlets were transferred to modified *R* medium of Power (1987).

RESULTS

The development of calli was influenced by a few factors: the age of the immature embryos, the genotype and the media. Another important factor for the donor plants was their growth environment which was the field.

The optimal age for embryo development was determined to be 8-10 days. Excised embryos placed on initiation medium produced calli during two weeks of *in vitro* culture (Table 2). Visible changes were registered after 10 days when the embryo surface became irregular and transformed into callus. The results presented in Table 2 show that genotype 2607 x E045 demonstrated the highest response, while the genotype 2607 x Verb. the lowest one. The regenerable type of callus was yellowish and contained green buds. The friable calli were white, resulting in dedifferentiated tissue without the possibility for organized tissue. The best result - organized structures - was observed on a medium containing 2 mg/l zeatin (Figure1). It is well-known that zeatin is one of the best growth regulators for regeneration. In our experiments, regeneration was induced by transferring the calli to the same medium. High sucrose concentrations did not have a significant positive influence on shoot formation, therefore, we used initial media with a very low sucrose concentration (Figure 2).

Table 2: Callus formation and plant morphogenesis of different genotypes

Genotype	Callus formation (%)				Plant morphogenesis (%)			
	K	Z1	Z2	Z3	K	Z1	Z2	Z3
1234xE045	42.4	44.4	37.1	51.8	9.1	44.4	47.5	44.4
1607xE045	12.5	11.1	5.8	6.2	12.5	5.5	11.7	12.5
2607xE045	75.0	50.0	75.0	25.0	50.0	0	50.0	25.0
2607xVerb.	0	0	3.3	0	0	0	0	0

As our previous data showed, the supplements were important for indirect organogenesis. It appeared that the kind of basic mineral medium used (*Gambourg* or *MS*), was not important but the type and concentration of plant growth regulators was found to be the limiting factor (unpublished data).

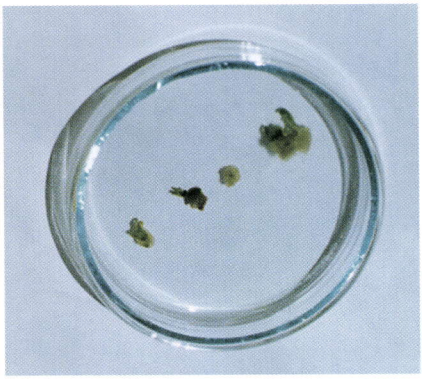

Figure 1: The influence of different hormones on genotype 1607xE045. From left to right: Z3, K, Z1 and Z2

Figure 2: Genotype 2607xE045 on MS medium with low sucrose gradient, supplemented with 2 mg/l zeatin. Different stages: from embryo derived callus to shootlets

Distinct differences in regenerable calli production were observed among different genotypes. These differences in regeneration response indicated the important influence of the parental genotype.

The formation of roots was a serious problem for some regenerants, in spite of the efforts to stimulate root induction. Within 20-30 days they developed 5-6 leaves and roots. Some regenerants were morphologically abnormal with thin leaves and "bottle-shaped" stems. There was no correlation between the type and concentration of growth regulator used and the abnormality observed. The most responsive genotype was 2607 x E045 which had a regeneration capacity of 75%, 1607 x E045 and 1234 x E045 genotypes were the least responsive, and 2607 x Verb. was not capable of regeneration.

DISCUSSION

We have showed that under appropriate culture conditions morphogenetic calli could be produced from immature embryos of some sunflower genotypes. Factors influencing the expression of totipotency in tissue culture are genotype, plant growth regulator and embryo age. Callus initiation and morphogenetic capacity were obtained with zeatin.

The type of plant growth regulator has an important effect on callus formation and plant regeneration. Somatic embryogenesis from immature embryos has been obtained by different authors using NAA, BAP, GA_3 - Paterson and Everett (1985), 2-4-D-abscisic acid – Wilcox-McCann *et al.* (1988), zeatin - Li *et al.* (1988). Zeatin in combination with a low sucrose concentration and a combination of macro- and micro-elements of *MS* medium proved to be excellent for callus initiation.

The genotype also has a large effect on plant regeneration. Chandler and Beard (1983) developped a reliable system for regeneration potential with 53 genotypes. Evidence for genetic control *in vitro* was obtained from Finer (1987) and Wilcox *et al.* (1988). The fact that some genotypes produced calli and regenerated plants while others were not capable of it indicates that there must be some important gene(s) (Paterson and Everett, 1985). Knitel *et al.* (1991) suggested the possibility for genetic determination of this trait. Studies to determine the genetic basis of regeneration potential are in progress.

CONCLUSIONS

Plant regeneration from immature embryos of 2607 x E045, 1607 x E045 and 1234 x E045 were obtained. The best regeneration media was MS, NAA 0.5 mg/l; Zeatin 2.0 mg/l, sucrose 5000 mg/l. The best genotype was 2607 x E045. Further improvement of the regeneration system is needed, studying additional parameters that influence the regenerative response of immature embryos.

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REGENERACIÓN POTENCIAL DE HÍBRIDOS DE GIRASOL

RESUMEN

La regeneración potencial de plantas de dos híbridos F_1 de girasol fue evaluada. Los híbridos inmaduros (8-10 días de edad) fueron recolectados. El medio basal MS suplementado con 2 mg/ml de zeatina fueron mejores que otros variantes testados pare iniciación y regeneración de cello. Los cellos regenerados fueron obtenidos de ambos híbridos. Las plántulas completes se formaron en 20-30 días. Parte de las plantas regeneradas no mostraron alteracionesmayores en morfología en comparación con los originales.

POTENTIEL DE RÉGÉNÉRATION DES HYBRIDES DE TOURNESOL

RÉSUMÉ

Le potentiel de régénération de deux hybrides F_1 de tournesol a été évalué. Des embryons immatures (âgés de 8-10 jours) ont été récoltés. Un milieu de base de type MS complété par 2 mg/l de zéatine, s'est révélé meilleur que les autres variantes testées pour l'initiation de cals et la régénération. Des cals aptes à la régénération vent obtenus à partir des deux hybrides. Des plantes complètes vent formées en 20-30 jours. Les plantes régénérées ne montrent aucune altération majeure de morphologie par rapport aux plantes originales.