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SUNFLOWER GENOTYPE REACTION TO DIRECT AND INDIRECT ORGANOGENESIS AND SOMATIC EMBRYOGENESIS USING THREE MEDIA AND GAMMA RAY TREATMENT

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SUMMARY

Immature zygotic embryos from nine inbred lines of sunflower (*Helianthus annuus* L.) were used as a donor material for the induction of direct organogenesis and somatic embryogenesis. A portion of the embryos was treated with Cs 137 gamma radiation before plating, at doses of 5, 10 and 15 Gy.

Three induction media were used in the experiments - A0 by Freyssinet and Freyssinet (1988), E1 (modified A0 medium) and TPM by Wilcox *et al.* (1988).

The regeneration was strongly increased with the dose of 5 Gy in line Z-8-A in the medium A0, followed by the dose of 10 Gy in the lines 1395 Rf in A0 and RNA-801 in E1. For the three media and all genotypes the dose of 5 Gy in the medium A0 appear to be most appropriate for increasing the frequency of plant regeneration.

INTRODUCTION

Plant regeneration by direct and indirect organogenesis and somatic embryogenesis in tissue culture of sunflower (*Helianthus annuus* L.) has been reported by several authors (Paterson and Everett, 1985; Finer, 1987; Freyssinet and Freyssinet, 1988; Witrzens *et al.*, 1988; Wilcox *et al.*, 1988; Khnittel *et al.*, 1991; Pugliessi *et al.*, 1991).

There are few publications about combined use of mutagenesis and *in vitro* culture.

Mutagenesis, both physical and chemical, has proved favorable for mutation induction in tissue culture (Skirvin, 1978; Novak *et al.*, 1988; Cheng *et al.*, 1990 and Encheva *et al.*, 1993).

We report here the influence of three doses of gamma radiation 5, 10 and 15 Gy, for increasing the frequency of plant regeneration *in vitro*.

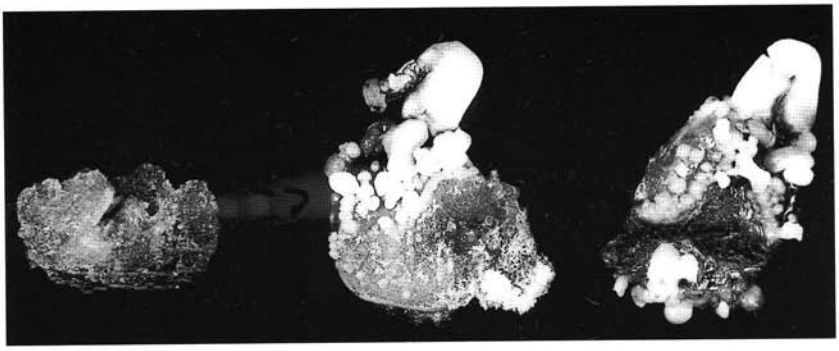

Figure 1. Somatic embryogenesis on medium TPM

Figure 2. Direct organogenesis on medium A0

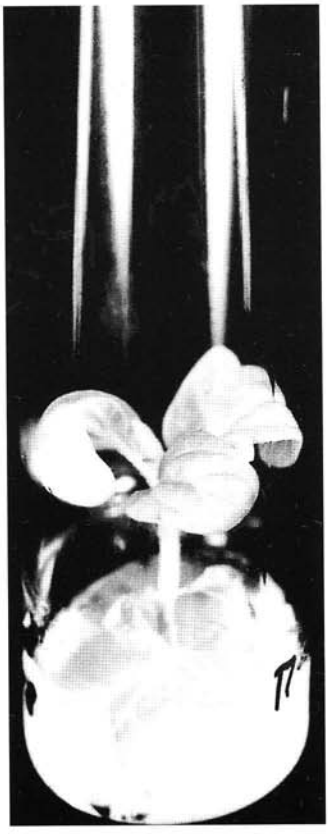

Figure 3. Plant regeneration on medium E1

MATERIALS AND METHODS

The experiments were performed on nine highly lines uniform from the collection of the institute. Donor plants were grown under field conditions during 1992-1993. The plants were hand pollinated and 7-18 days (2-8 mm) later immature zygotic embryos were collected at cotyledon stage. We used more frequently immature embryos 7-14 days (2-6 mm) after pollination.

A portion of the embryos was treated with Cs 137 gamma radiation before planting, with doses of 5, 10 and 15 Gy. The dose of 15 Gy was used in 1992 only and the results are included in Table 2. Immature zygotic embryos were surface sterilized by stirring in a 2.7% commercial bleach solution for 20 min and then rinsed 3 times in sterilized distilled water. Aseptically dissected immature embryos were plated on three induction media and cultured for 2-3 weeks in the dark at 25-26°C.

In our experiments we used three induction media - for direct organogenesis and somatic embryogenesis - A0 by Freyssinet and Freyssinet (1988) and E1 (modified A0 medium without aminoacids and decreased concentracion of sucrose). The third medium, TPM, is for indirect organogenesis and somatic embryogenesis by Wilcox *et al.*, (1988).

Isolated adventitious buds and somatic embryos were transferred to media for growing and rooting by Wilcox *et al.*, (1988).

RESULTS

Table 1: Responsiveness of some genotypes to culturing *in vitro* on three media combined with gamma ray treatment [produced regenerants for 1992-1993 (%)]

Genotype	Medium								
	A0			TPM			E1		
	C	5 Gy	10 Gy	C	5 Gy	10 Gy	C	5 Gy	10 Gy
L-2128	31.30	45.84	28.21	11.10	3.34	5.56	54.80	30.66	30.65
RNA-857	3.71	6.88	0	2.22	7.10	1.11	12.40	7.90	18.00
K-821	0	30.00	0	0	0	0	7.69	25.00	7.69
R-20	33.34	12.86	2.04	1.25	1.74	1.11	15.50	7.70	4.39
RNA-801	0	0	0	14.40	0	0	30.00	15.52	60.72
1028 Rf	11.11	6.25	6.00	3.34	0	0	0	0	0
1395 Rf	0	12.07	31.82	7.78	0	4.45	9.26	13.64	0
Z-8-A	0	100.00	0	0	0	0	12.10	7.15	0
147 Rf	0	0	0	28.60	14.59	4.17	15.30	20.84	0
Total	8.83	23.74	7.56	7.64	2.97	1.82	17.40	14.27	13.46

The data in Table 1 indicated that the dose of 5 Gy has a pronounced, stimulating effect on the frequency of plant regeneration in five cultured lines in the medium A0, while the dose of 10 Gy manifests an inhibitory effect in all investigated geno-

types, except the line 1395 Rf. Gamma treatment with the dose of 5 Gy strongly increased regeneration frequency in the line Z-8-A in the medium A0, followed by the line 1395 Rf.

The line 1395 Rf showed the best responsiveness to *in vitro* culturing combined with gamma treatment with both doses 5 and 10 Gy.

In contrast, plant regeneration was not observed in the lines RNA-801 and 147 Rf in the three investigated variants.

The results of the treatment with 15 Gy (Table 2) showed that this dose increased regeneration frequency in the line L-2128.

Table 2: Responsiveness of some genotypes to culturing *in vitro* on three media combined with gamma ray treatment [produced regenerants for 1992 (%)]

Genotype	Medium					
	A0		TPM		E1	
	C	15 Gy	C	15 Gy	C	15 Gy
L-2128	62.59	100.00	0	0	89.69	25.30
RNA-857	7.41	14.29	0	0	11.63	13.33
K-821	0	0	0	0	0	0
R-20	66.67	2.00	2.50	0	31.03	1.49
RNA-801	0	0	28.95	0	0	0
1028 Rf	22.22	0	0	0	0	0
1395 Rf	0	0	0	0	18.52	0
Z-8-A	0	0	0	0	11.76	0
147 Rf	0	0	41.67	0	0	0
Total	17.65	12.92	8.12	0	18.07	4.46

The modified E1 medium with the dose of 5 Gy shows a stimulating effect in the three lines. The dose of 10 Gy increased regeneration frequency in the lines RNA-801 and RNA-857, while for all other lines the stimulation was decreased or was not noticed. Lack of regeneration in all three variants was observed in the line 1028 Rf only.

The dose of 15 Gy stimulated the line RNA-857 to a smaller degree. The behavior of genotypes to the medium for indirect organogenesis and somatic embryogenesis - TPM, was considerably different. Only the dose of 5 Gy with the line RNA-857 showed a stimulating effect about frequency of regeneration, while the doses of 5 and 10 Gy strongly inhibited the reaction of the other genotypes.

The lines RNA-801 and 1028 Rf did not react *in vitro* to both doses, 5 and 10 Gy.

Lack of regeneration *in vitro* in the third variant was observed in the lines K-821 and Z-8-A.

In contrast to the other two medium, A0 and E1, a stimulating effect of the dose of 5 Gy, with all investigated genotypes, was not observed in the medium TPM.

The highest regeneration frequency is demonstrated by the dose 5 Gy in the line Z-8-A in the medium A0, followed by 10 Gy in the line 1395 in the medium A0 appears to be most suitable for increasing frequency of plant regeneration. The lines 1395 Rf and K-821 proved to be pliable to gamma ray treatment.

DISCUSSION

There are few publications about somaclonal and mutation induced variation (Skirvin *et al.*, 1978; Gao *et al.*, 1986; Novak *et al.*, 1988; Cheng *et al.*, 1990 and Encheva *et al.*, 1993).

Positive results were obtained when mutagenesis and tissue culture were combined in appropriate ways in wheat, maize and sunflower (Novak *et al.*, 1988; Cheng *et al.*, 1990; Encheva *et al.*, 1993).

It appears that the dose of 5 Gy is suitable for the reduction of plant height in maize (Novak *et al.*, 1988) and also to change many characteristics in wheat (Cheng *et al.*, 1990). Encheva *et al.*, (1993) reported that the dose of 7 Gy is more effective than 10 Gy for many characteristics (date of flowering, plant height, head diameter, 1000-seed weight, oil in the kernel and fatty acid composition of sunflower). There are insufficient, contradictory and incomplete investigations about influence of gamma treatment on increasing frequency of plant regeneration. Gao *et al.*, (1986) reported a stimulating effect of gamma radiation and *in vitro* culturing on the frequency of plant regeneration.

Novak *et al.*, (1988) described a significant decrease of regeneration frequency *in vitro* as a result of gamma tratment in maize.

Encheva *et al.*, (1993) initiated a stimulating effect of the doses of 7 and 10 Gy in increasing plant regeneration in two sunflower genotypes.

Our additional investigation in nine highly uniform lines of sunflower confirmed a stimulating effect of gamma radiation in the doses of 5 and 10 Gy on increasing the frequency of plant regeneration. The results showed that there is a close interaction between genotypes and different doses of gamma ray treatment.

An interaction was observed between composition of nutrition medium and the dose of radiation. Stimulating effect of gamma treatment is most obvious with the dose of 5 Gy in the medium A0 for direct organogenesis and somatic embryogenesis, while with the medium TPM the percentage of regeneration is rather low for all investigated variants.

Besides positive results about production of mutagen-induced genetic variation, gamma treatment combined with tissue culture to an increase of the frequency of plant regeneration in sunflower.

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REACCIÓN DE GENOTIPOS DE GIRASOL A ORGANOGÉNESIS DIRECTA E INDIRECTA Y A EMBRIOGÉNESIS SOMÁTICA UTILIZANDO TRES MEDIOS Y TRATAMIENTO DE RAYOS GAMMA

RESUMEN

Embriones zigóticos inmaduros de nueve líneas puras de girasol (*Helianthus annuus* L.) fueron usados con material donante para la inducción de embriogénesis directa e indirecta y embriogénesis somática. Una porción de los embriones fueron tratados con radiación gamma Cs 137 antes de la siembra con dosis de 5, 10 y 15 Gy, respectivamente.

En el experimento fueron usados tres medios A0 (Freyssinet y Freyssinet, 1988), E1 (medio A0 modificado) y TPM (Wilcox et al., 1988).

La regeneración fue incrementada fuertemente a la dosis de 5 Gy en la línea Z-8-A en el medio A0, sequida por dosis de 10 Gy en líneas 1395 Rf en A0 y RNA-801 en E1. Para los tres medios y todos los genotipos la dosis de 5 Gy en el medio A0 parece ser el más apropiado para incrementar la frecuencia de regeneración de plantas.

RÉPONSE À L'ORGANOGÉNÈSE INDIRECTE ET À L'EMBRYOGÉNÈSE SOMATIQUE DE GÉNOTYPES DE TOURNESOL CULTIVÉS SUR TROIS MILIEUX ET SOUMIS À UNE IRRADIATION GAMMA

RÉSUMÉ

Des embryons immatures zygotiques issus de neuf lignées fixées de tournesol (*Helianthus annuus* L.) ont été utilisés comme matériel de départ pour l'induction directe ou indirecte de l'organogénèse et de l'embryogenèse somatique. Une fraction des embryons a été traitée par rayonnement gamma du Cs 137 à des doses 5, 10 et 15 Gy, avant mise en culture. Les trois milieux de culture inducteurs suivants ont été utilisés: A0 de Freyssinet et Freyssinet (1988), E1 (milieu A0 modifié) et TPM de Wilcox et al. (1988). La régénération a été fortement augmentée avec une dose de 5Gy chez la lignée Z-8A dans le milieu A0, suivie par le traitement à la dose de 10 Gy chez les lignées 1395 Rf dans AO et RHA-801 dans E1. Pour les trois milieux et l'ensemble des génotypes, la dose de 5 Gy sur le milieu A0 paraît la plus appropriée pour augmenter la fréquence de régénération des plantes.