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RAPID CYCLING THROUGH IMMATURE EMBRYO CULTURE IN SUNFLOWER (Helianthus annuus L.)

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SUMMARY

Attempts were made in sunflower to develop plants from immature embryos instead of planting seed. Immature embryos of 12 days after flowering were dissected from seed-grown plants (SGP) and 1st cycle of embryo-raised plants (ERP) was obtained. When these plants were at flowering stage, 12-day old embryos were dissected and 1nd cycle of ERP was raised. This procedure was followed for 5 cycles. Through this procedure, within 316 days, 5 cycles of ERP were completed. No variation was observed in plants of all 5 cycles of ERP except for male sterility. This methodology could be useful in rapid development of inbred lines, fast conversion of an inbred to cytoplasmic male sterile line or for any backorossing programme as compared with seed-to-seed cycle. Such rapid cycling of successive generations within short period helps to achieve homozygosity early, which would accelerate the breeding cycle of crop plants.

Key words: Sunflower, immature embryo, seed-grown plant (SGP), embryo-raised plant (ERP), cytoplasmic male sterility (CMS).

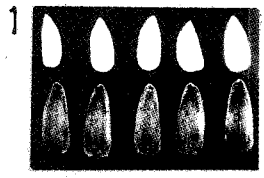
INTRODUCTION

Sunflower is a new crop in India and the area under cultivation is increasing rapidly. It is a highly cross-pollinated crop and development of homozygotes is important both for genetic studies and hybrid seed production. Conventional methods require a minimum of 6 generations to develop nearly homozygous lines. Completion of several successive generations within a short period of time can facilitate genetic improvement of crop plants (Williams and Hill, 1986). Anther culture is commonly employed to develop homozygosity and thus help in shortening the breeding cycle for hybrid development. However, in sunflower, this approach bas not been exploited commercially (Yang et al., 1990). In the present study, attempts were made for a rapid generation advancement through immature embryo culture.

MATERIAL AND METHODS

The dwarf and early maturing variety Morden was used in the present study. The entire work was carried out between June 1992 and July 1993.

Medium: Murashige and Skoog (1962) with 2% sucrose and 0.8% agar at pH 5.6-5.7 was used.



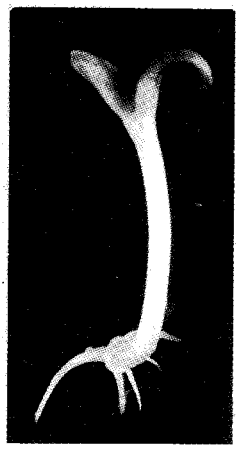


Figure 1 Twelve-day old achenes (bottom) and embryos (top)
Figure 2 Three-day old plantlet
Figure 3 Established plants in pot at flower bud stage

Emasculation and pollination: emasculation was done early in the morning at a stage when 2 to 4 whorls of flowers had opened. All flower buds in the centre of the head were removed. Pollination was carried out next day to ensure uniformity of embryo age.

Embryo culture: achenes were removed from the head on the 12th day after pollination. They were sterilized with 0.1% mercuric chloride for 5 minutes and sodium hypochlorite: water (1:9) solution for 10 minutes. Subsequently, 3 washings 5 minutes each with sterile distilled water were made. Embryos were dissected and transferred to the medium. All operations were carried out under aseptic conditions.

Hardening: after 3 days, welldeveloped plantlets were removed from the medium. Roots were gently and thoroughly washed in sterilized distilled water. The plantlets were transferred to a sterile mixture of sand: soil : vermiculite (1:1:1 v/v). They were kept at 25°C for 5 days under continuous illumination and then transferred to pots filled with soil, keeping 2-3 plants per pot.

RESULTS AND DISCUSSION

Seed-grown plants (SGP) flowered in the field 50 days after sowing and matured in 86 days indicating the minimal requirement of 86 days for completing the seed-to-seed cycle. Twelve days old embryos of SGP (Figure 1) were dissected and inoculated on the medium. At inoculation date, SGP had 64 days, while the embryoraised plants (ERP)

Table 1. Percent success of embryo-raised plants till maturity in five cycles.

Development stage	Cycles of embryo-raised plants					Mean ± S.D.
	1	2	3	4	5	
No.of embryos inoculated	100	88	96	96	96	92.5±3.91
No.of plantlets for hardening	80	72	63	72	80	77.4±6.31
	(80.0%)	(81.8%)	(65.6%)	(75.0%)	(83.3%)	(77.1%)
No.of plantlets in pots	61	68	48	44	55	55.2±8.65
	(61.0%)	(77.3%)	(50.0%)	(45.8%)	(57.3%)	(58.0%)
No.of established plants	47	59	33	39	43	44.2±8.70
Percentage of success	47.0%	67.0%	34.4%	46.6%	44.8%	46.4%

started their 1st day of the 1st cycle. Small roots and hypocotyl along with cotyledonary leaves appeared 3 days after inoculation (Figure 2). After hardening they were transferred to the field. The period between embryo inoculation and plantlet transfer to the pot was 8 days. At the time of harvest of SGP, ERP from the 1st cycle have initiated flower buds (Figure 3). They flowered in 50 days. Following pollination, 12-day old embyos were dissected and inoculated on the medium. At 65 days of the 1st cycle of ERP, the 2nd cycle of ERP was initiated. Thus, instead of waiting up to maturity, embryos were dissected

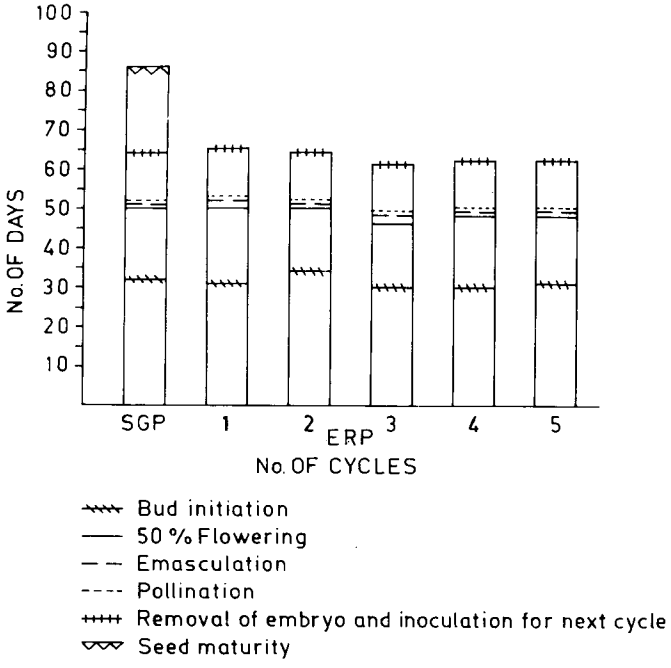


Figure 4. Developmental stages of seed grown plant (SGP) and embryo raised plant (ERP) from sawing/inoculation to seed maturity

and plants of 5 cycles were raised from 12-day old immature embryos. It is evident from the data that within 316 days, 5 cycles of ERP were completed (Figure 4). In comparison with SGP, no variation could be observed in ERP except for 1 male sterile plant the first 3 cycles. Gopalkrishnan (1993) suggested the method of shortening the breeding cycle by inducing adventitious buds in the hypocotyl region of immature embryos. This method takes 105 days from culture initiation to mature seeds. However, the vegetative phase of embryo-derived plants was very short resulting in small plant (12-25 cm), flower buds and early flowering.

Freshly harvested sunflower seeds remain dormant for 45-60 days (AICORPO report 1993) and this widens the gap between 2 generations. When embryos were dissected and grown on the medium, no dormancy was observed and thus considerable time was saved. Survival of the established plants till maturity was about 50% (Table 1). Wu et al. (1991) suggested that this technique could be useful in plant breeding. However, the number of generations that could be completed within a year is not clear. They used 2 modified MS media along with growth hormones. The present study shows that the MS medium without hormones is sufficient to raise plants from 12-day old immature embryo, which could easily be hardened in the sand: soil: vermiculite base. Williams and Hill (1985) reported that rapid cycling of *Brassica* could produce 6 to 10 generations per year and being a model plant in genetic and molecular biology studies.

In hybrid development programme, conversion of an inbred to a cytoplasmic male sterile (CMS) line is important. Any inbred line could be converted completely in CMS line by 6 backcrosses. Attempting 6 backcrosses through the seed-to-seed cycle is time consuming. The present study shows that in sunflower it is possible to advance 5 generations within a year without resorting to the seed-to-seed cycle. This could be of great importance in breeding especially for fast conversion of an inbred to a cytoplasmic male sterile line.

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ACELERACION DE CICLOS A TRAVES DE CULTIVO DE EMBRIONES INMADUROS EN GIRASOL (*Helianthus annuus* L.)

RESUMEN

Fueron hechas pruehas en girasol para desarrollar plantas procedentes de embriones inmaduros en vez de semilla. Los embriones inmaduros 12 días después de la floración fueron rescatados de semillas en crecimiento de plantas (SGP) y el primer ciclo de plantas procedentes de embriones (ERP) fue obtenido. Cuando estas plantas estuvieron en el estado de floración embriones de 12 días fueron rescatados y el segundo ciclo fue alcanzado. Este procedimiento fue seguido durante cinco ciclos. A través de este procedimiento, dentro de 316 días, cinco ciclos de plantas procedentes de embriones (ERP) fueron complatados. Ne se abservó variación en plantes en el ciclo 5° de plantas ERP excepto para androesterilidad. Esta metodología podría ser utílizada en el desarrollo rápido do líneas puras, rápida conversión de una línea a androesterilidad citoplásmica o para programas de retrocruzamento comparado con ciclos de semilla a semilla. Esta aceleración de ciclos de generaciones sucesivas dentro de un corto perlado ayuda a alcanzar la homocigosis en el periodo mas corto, 10 que podría acelerar el ciclo de mejora de las plantas.

ACCELERATION DES CYCLES PAR CULTURE D'EMBRYONS IMMATURES DE TOURNESOL (*Helianthus annuus* L.)

RÉSUMÉ

Des essais ont été réalisés chez le tournesol pour obtenir des plantes par culture d'embryon immatures à la place du semis de graines. 12 jours après la floraison les embryons sont disséqués à partir de plantes issues de graines (SGP) pour produire le 1°r cycle d'embryoculture (ERP). Lorsque ces plantes sont en phase de floraison, les embryons êgés de 12 jours sont disséqués à leur tour pour produire le 2° cycle d'embryons (ERP). Ce processus est reproduit durant 5 cycles. A l'aide de cette procédure, 5 cycles d'ERP sont réalisés en 316 jours. On n'a pas observé de variation sur les plantes au cours des 5 cycles d'ERP si ce n'est pour la stérilité mêle. Par rapport à la méthode graine à graine conventionnelle, cette méthodologie peut être utile pour le développement rapide de lignées inbred, la reconversion rapide de lignées inbred en lignées mâle stériles cytoplasmiques ou pour tout programme de retrocroisement. Cette succession rapide de générations dans une courte période facilite l'obtention de l'homozygotie dans les courts délais, ce qui devrait permettre d'accélérer le cycle de sélection des plantes cultivées.