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SHOOT ORGANOGENESIS FROM COTYLEDONS OF SUNFLOWER

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SUMMARY

The experiment was conducted in order to study the regeneration potential of cotyledon explants from some Iranian inbred lines of sunflower (*Helianthus annuus* L.) and their F_1 hybrids. Cotyledons of 2-day-old seedlings of three cytoplasmic male sterile inbred lines used as female (CMS 24, CMS 31 and CMS 60/52) and five inbred lines used as male (R-81.1, R-92, R-97, R-201 and R-232) and finally their 15 F_1 hybrids were plated on shoot induction medium, Murashige and Skoog (MS) containing 3% sucrose, 0.5% agar-agar and different concentrations of BAP and NAA. Analysis of variance showed significant differences for all organogenesis traits studied among parental genotypes and F_1 hybrids, hormone combination treatments and genotype × hormone combination treatment interaction. Shoot organogenesis was optimized on MS medium with 1 mg l^{-1} BAP and 1 mg l^{-1} NAA.

Interaction "(CMS 24 × R-201) × T2" showed the highest values for number of explants shooting per 100 explants plated (55%). Interaction "R-92× T2" showed the highest value for average number of shoots per explants plated (8.65). Interaction "R-81.1 × T2" showed the highest value for average number of shoots per explants shooting (23). Interaction "CMS 24 × T2" showed the highest value for number of explants rooting per 100 explants plated (35%).

Key words: cotyledon, *Helianthus annuus*, plant regeneration, rooting, sunflower

INTRODUCTION

Sunflower is one of the most important oil crops. Its genetic improvement for several traits is necessary. Wild species of sunflower can be used as genetic sources of valuable traits such as pest and disease resistance and tolerance to salt and drought stresses. The use of many wild species in breeding programs is restricted

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by natural barriers. New techniques in biotechnology such as somatic hybridization or gene transfer methods can be utilized for overcoming those obstacles. Somaclonal variants can be also used as new plant genetic resources (Fiore *et al.*, 1997). In more cases, success in the mentioned techniques depends on success in plant regeneration from different explants such as cotyledons, hypocotyl, epiderm and leaf (Laparra *et al.*, 1997).

The application of biotechnology for the improvement of sunflower has been limited due to difficulties associated with the lack of an efficient and reproducible method for plant regeneration (Ceriani *et al.*, 1992). Sunflower has been regenerated by organogenesis (Power, 1987; Wirtzens *et al.*, 1988; Chraibi *et al.*, 1991) or somatic embryogenesis (Pelissier *et al.*, 1990). Successful regeneration from de-differentiated tissues arising from explants has been reported, such as from thin cell layers (Pelissier *et al.*, 1990; Bolandi *et al.*, 2000), cotyledons (Knittel *et al.*, 1991; Chraibi *et al.*, 1992; Deglene *et al.*, 1997; Baker *et al.*, 1999), leaf, rhizome and stem explants (Laparra *et al.*, 1997). Regeneration frequency depends on genotype, for which most genotypes are reported recalcitrant (Wirtzens *et al.*, 1988) as well as for other factors such as the nature and developmental stage of the explants (Baker *et al.*, 1999), plant growth regulators (Pugliesi *et al.*, 1993) and regeneration method (Chraibi *et al.*, 1992). Cotyledons of mature seeds are a frequent source of explants for organogenic regeneration, in part because of their year-round availability, ease of culture initiation and applicability to a number of genotypes (Baker *et al.*, 1999).

Unfortunately, researches pointed out that many genotypes exhibited difficulties in plant regeneration (Power, 1987; Wirtzens *et al.*, 1988). Modifications in *in vitro* environmental factors such as growth regulators can potentially improve plant regeneration in these genotypes. Both NAA and BA must be present for organogenesis of mature and immature sunflower cotyledons (Baker *et al.*, 1999).

In this paper we presented results on the regeneration potential of cotyledon explants from some Iranian inbred lines and their hybrids using the shoot induction medium with different concentrations of BAP and NAA and studying the rooting with different concentrations of GA_3, NAA and IAA.

MATERIALS AND METHODS

Three cytoplasmic male sterile inbred lines were used as females (CMS 24, CMS 31 and CMS 60/52) and five inbred lines used as males (R-81.1, R-92, R-97, R-201 and R-232) and finally their 15 F_1 hybrids were studied. The experimental design was factorial on the basis of complete randomized block design with 3 replications per treatment. Each replication consisted of 6 petri dishes (55×15 mm) of 4 explants. The first factor was 23 genotypes and the second factor was three hormonal combination in shoot induction medium. To provide plant materials prior to culturing, on the medium, pericarps were removed and seeds surface sterilized for

20 min in a solution of 2% (w/v) sodium hypochlorite (NaClO) and then washed three times in sterile distillated water.

Seeds were germinated in culture tubes on hormone-free half strength Murashige and Skoog's (MS) medium containing 0.1% vitamine, 2% sucrose and 0.5% agar-agar. The pH was adjusted to 5.6 before autoclaving at 120°C for 20 min. Seeds were cultured in test tubes each containing 10 ml of germination medium. Cultures were incubated in a growth chamber with a photoperiod of 16 h and temperature of 25°C.

Cotyledons of 2-day-old seedlings were excised and cut transversely into two pieces. Four explants from each seedling were then plated on shoot induction medium. This medium consisted of full strength MS medium containing 3% sucrose, 0.5% agar-agar, 0.05% casein hydrolyzate and different levels of BAP and NAA, as follow:

T1: 1.2 mg l^{-1} BAP + 0.82 mg l^{-1} NAA,

T2: 1 mg l^{-1} BAP + 1 mg l^{-1} NAA,

T3: 1 mg l^{-1} BAP + 0.52 mg l^{-1} NAA.

The pH was adjusted to 5.8 before autoclaving cotyledon culture was performed in 55 × 15 mm plastic petri dishes containing 8 ml of shoot induction medium and petri dishes were sealed with parafilm. Cultures were maintained at 25°C and 16/8 (day/night) photoperiod. After 15 days, the following four traits were studied (shoots > 4 mm were counted):

- number of explants shooting per 100 explants plated (ES/100EP),
- average number of shoots per explants plated (shoot/EP),
- average number of shoots per explants shooting (shoot/ES),
- number of explants rooting per 100 explants plated (ER/100EP).

Shoots were transferred to test tubes containing elongation medium, hormone free MS with pH adjusted to 5.6. For rooting, shoots were transferred to half strength MS containing 0.05% casein hydrolyzate, 1.5% sucrose, 2.5 g l^{-1} activated charcoal and 0.5% agar-agar and three hormonal treatments as follow:

T1: No hormons,

T2: (0.5 mg l^{-1}) NAA+(0.5 mg l^{-1}) GA_3,

T3: (0.5 mg l^{-1}) IAA + (0.5 mg l^{-1}) GA_3.

The pH was adjusted to 5.7 before autoclaving.

RESULTS AND DISCUSSION

Analysis of variance showed significant differences for all organogenesis traits studied among parental genotypes and F_1 hybrids, hormone combination treatments and genotype×hormone combination treatment interaction (data not showed). Genotype means for organogenesis parameters are summarized in Table1 .

Table 1: Mean performance for organogenesis parameters in inbred lines and F_1 hybrids

Genotype	ES/100EP	Shoot/EP	Shoot/ES	ER/100EP
CMS 24	11.1 fg	1.3 cd	10.3 bc	24.4 a
CMS 31	13.9 ef	0.8 defgh	6.1 de	10.6 bc
CMS 60/52	25.4 bc	2.3 b	11.1 b	3.9 fghi
R-81.1	20.6 cd	1.6 c	10.7 bc	5.6 efg
R-92	35 a	5.2 a	12.8 ab	15 b
R-97	1.6 k	0.0 l	0.3 l	3.9 efghi
R-201	16.3 de	2.2 b	13.5 a	0.0j
R-232	5.3 j	0.7 ghijk	4.9 h	0.0j
CMS 24 × R-81.1	15.4 ef	1.0 defgh	3.7 gh	8.9 cd
CMS 24 × R-92	5.3 j	0.4 jk	5.4 fgh	6.7 cdef
CMS 24 × R-97	9.9 gh	0.6 fghij	4.3 fgh	0.6 ij
CMS 24 × R-201	26 bc	2.8 b	6.6 def	1.7 ghij
CMS 24 × R-232	12.9 fg	0.6 fghij	4.6 efgh	3.3 fghi
CMS 31 × R-81.1	26.2 b	1.6 c	5.9 de	4.3 efgh
CMS 31 × R-92	15.8 de	0.9 defg	5.2 def	3.3 fghi
CMS 31 × R-97	5.3 j	0.2 kl	1.8 l	0.6 ij
CMS 31 × R-201	14.2 ef	1.1 de	5.7 defg	4.4 efg
CMS 31 × R-232	12.5 fg	1.1 def	6.1 def	2.2 ghij
CMS 60/52 × R-81.1	14.2 ef	0.7 efghi	7.3 cd	7.6 cde
CMS 60/52 × R-92	11.8 fgh	1.4 cd	8.2 de	2.1 ghij
CMS 60/52 × R-97	1.6 k	0.0 l	0.6 i	1.1 hij
CMS 60/52 × R-201	8.5 hi	0.5 hijk	4.0 fgh	1.6 ghij
CMS 60/52 × R-232	6.3 ij	0.4 ijk	5.4 efgh	6.1 def

Means followed by similar letters in each column are not significantly different at 1% level according to Duncan' s Multiple Range Test

Inbred line R-92 and F_1 hybrid CMS 31 × R-81.1 presented the highest values for number of explants shooting per 100 explants plated (35 and 26.2%, respectively).

Three inbred lines R-92, CMS 60/52 and R-201 and F_1 hybrid CMS 24 × R-201 presented the highest values for average number of shoots per explants plated (5.19, 2.31, 2.21 and 2.82, respectively).

Three inbred lines R-201, R-92 and CMS 60/52 presented the highest values for average number of shoots per explants shooting (13.2, 12.78 and 11.05, respectively). Three inbred lines CMS 24, R-92 and CMS 31 presented the highest values for number of explants rooting per 100 explants plated (24.44, 15 and 10.55, respectively). A significant genetic variation for organogenesis parameters was observed as has been reported previously by Power (1987), Espinasse *et al.* (1989), Sarrafi *et al.* (1996 a, b).

Table 2 shows that the hormone combination treatment T2 had the highest value for number of explants shooting per 100 explants plated (18.49), average number of shoots per explants plated (1.85) and average number of shoots per explants shooting (9.16). Treatments T1 and T2 had the highest values for number of explants rooting per 100 explants plated (6.99 and 6.23, respectively). These results show that shoot organogenesis was optimized on MS medium with 1 mg l^{-1} BAP and 1 mg l^{-1} NAA and was similar to results reported by Deglene *et al.* (1997).

Table 2: Hormone combination treatments effect on organogenesis parameters

Treatment	ES/100EP	shoot/EP	shoot/ES	ER/100EP
T1: 1.2 mg l^{-1} BAP + 0.82 mg l^{-1} NAA	12.5 b	1.0 b	5.4 b	7.0 a
T2: 1 mg l^{-1} BAP + 1 mg l^{-1} NAA	18.5 a	1.9 a	9.2 a	6.2 a
T3: 1 mg l^{-1} BAP + 0.52 mg l^{-1} NAA	10.1 c	0.8 c	4.3 c	2.1 b

Means followed by similar letters in each column are not significantly different at 1% level according to Duncan' s Multiple Range Test

Interactions "(CMS24 × R-201) × T2", "CMS60/52 × T1", "R-92 × T2" and "R-92 × T3" showed the highest values for number of explants shooting per 100 explants plated (55, 52.76, 51.66 and 46.66, respectively), data of genotype × hormone combination treatment interactions not presented. Interactions "R-92 × T2", "(CMS24 × R-201) × T2" and "R-92 × T3" showed the highest values for average number of shoots per explants plated (8.65, 7.05 and 6.38, respectively). Interactions "R-81.1 × T2", "CMS24 × T1" and "R-92 × T2" showed the highest values for average number of shoots per explants shooting (23, 18.02 and 16.7, respectively). Interactions "CMS24 × T2", "R-92 × T1" and "CMS24 × T3" showed the highest values for number of explants rooting per 100 explants plated (35%, 26.7% and 26.7%, respectively). Finally, some healthy plantlets were obtained from this experiment.

From one hundred and twelve seedlings that have been produced in the tube test about 50% were normal and the rest exhibited premature flowering, vitrification and callus formation. When we transferred seedling to rooting medium, our use of activated charcoal in rooting medium prevented callus formation, increased the percentage of seedling that were rooted and decreased the time required for rooting. Reducing the time that seedlings spent on rooting medium also reduced vitrification and sped up the transition to the greenhouse (Baker *et al.*, 1999; Chraibi *et al.*, 1992). Shoot elongation and development have been improved by using GA_3 (Power, 1987; Wirtzens *et al.*, 1988), although GA_3 tends to produce shoots that are thin pale and overelongated too (Paterson, 1984; Power, 1987). Low level (0.1 mg l^{-1}) GA_3 improved shoot elongation and at GA_3 concentration of 0.5 and 1.0 mg l^{-1} (high level) many shoots were thin and pale (Baker *et al.*, 1999).

In our experiment, many seedlings cultured in T2 and T3 media were thin and pale. We observed that the addition of GA_3 produced many seedling were thin and pale and did not survive for transplanting to soil.

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ORGANOGENESIS DE LA TALLOS COTILEDONES DE GIRASOL

RESUMEN

E experimento realizado por estudio la regeneración potential de explantes cotiledones a las líneas inbreda Irano de girasol (*Helianthus annuus* L.) y los híbridos F_1. Cotiledones de la semillas dos días la 3 lineas utilizado para maternal (CMS 24, CMS 31 y CMS 60/52) y cinco lineas inbredas paternal (R-81.1, R-92, R-97, R-201 y R-232) y 15 híbridos F_1 se transfirieron a un media de Murashige and Skoog (MS) agüé contiene 3% sucrose, 0.5% agar-agar y concentración diferentes BAP y NAA. E1 análisis de varianza ilustro diferentes significados para todos los rasgos oranogamesis genotipos paren tal y híbridos F_1, y combina tío tratamientos y combinación genotipo y hormaon organogenesis se optimo en la medio MS auge contiene 1 mg l^{-1} BAP y 1 mg l^{-1} NAA.

Interacción, "(CMS 24 x R-201) x T2", indico alta valscion por el numero de germoglio cada (55%), interacción "R-92 x T2" indico alta valuación para la mediano numero de la germoglio cada explants (8.65). interacción "R-81.1 x T2" indica la alta valuación para Mediano germoglio cada explanta germoglio (23). Interacción "CMS 24 x T2" indica la alta valuación para la numeros de explantas rooting cada 100 explantas (35%).

ORGANOGENESE A PARTIR DES COTYLEDONS DE TOURNESOL

RESUME

Une experimentación a été réalisée afin d étudier l'aptitude a la règèneration à partir de cotylédons de tournesol (*Helianthus annuus* L.). Letude porte sur trois lignées pures cytoplasme male stérile (CMS 24, CMS 31 y CMS 60/52), cinq lignées males (R-81.1, R-92, R-97, R-201 y R-232), et leurs 15 hybrides F_1. Les cotylédons prélevés a partir de graines germées et cultivés sur le milieu MS contenant 3% de saccharose, 0.5% d agar-agar et les différents concentrations de BAP et de NAA.

Les résultats d analyse de la variance montrent une différence significative parmi les génotypes, les différentes concentrations de BAP et de NAA et leurs interactions, pour tous les caractères étudies. L'utilisation de 1 mg l^{-1} de BAP et 1 mg l^{-1} de NAA a permis une augmentation des pousses formées sur les cotylédons.

L'interaction "(CMS24 x R-201) x T2" a présente le taux maximum (55%) pour le nombre de cotylédons donnant au moins une pouse/100 cotylédons cultivés. L'interaction "(R-92 x T2)" a présente le meilleur résultat (8.65) pour le nombre moyen de pousses formées. Pour le nombre moyen de pousse formées, cotylédons donnât de pousse, la plus important est obtenue par l'interaction "R-81 x T2" (23). En ce qui concerne le nombre cotylédons enracinées pour 100 cotylédons cultivés la valeur la plus fort (35%) a été obtenue par l'interaction "CMS24 x T2".