J Phytol Res 16(2) 199-202, 2003

RESPONSE OF AMC-1 MAIZE COMPOSITE FOR CALLUS INDUCTION AND REGENERATION FROM YOUNG LEAVES AND TASSELS

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For callus induction and regeneration studies, young leaf and immature tassels were used as explants obtained from maize var. AMC-1. Best callus response was obtained when 2 4-D 2mg/ 1 was used in different media (GP, MS and N6). Maximum callus weight (1.84 gm) was recorded in G. P. medium. Callus regeneration giving completely developed plantlets was successful in basal media fortified with 1 mg/1 KIN and 1 mg/1 IAA.

Keywords : Callus; Immature tassel; Regeneration; Young leaf.

Introduction

Crop improvement primarily depends upon the availity of genetic variability. And therefore on its efficient induction and utilization[1]. The biotechnological methods of crop improvement involve manipulations and selection at cellular or tissue level[2] and subsequent regeneration of transgenic plants from cultured cells. Maize crop is highly recalcitrant to *in vitro* techniques and a lack of reliable regeneration system limits possible biotechnological applications in it[3]. Natural variability in most of the plants has been mostly used by intensive breeding effects. Genetic and cytogenetic changes are frequently observed in plant regenerated from tissue culture[4], such variation termed as somaclonal variation may provide a useful source of genetic variability for crop improvement. An effiicient method of callus induction and differentiation from suitable explants in maize would help to take up steps in this direction. The present study reports possibility of inducing such somaclonal variability in locally developed maize composite.

Materials and Methods

For callusing and regeneration two explants viz. young leaf and immature tassels were obtained.

Callus induction from young leaves : Leaf segments (4-5 cm long) were exised, from aseptically grown 20 days old seedlings of AMC-1 maize composite. The outer leaves were removed and the basal 2-3 cm of the immature leaves were cut transversely in to segments of 4-5 mm length. These leaf segments were cultured on MS[5]. G.P.[6], Chu[7] basal media supplemented with different levels of 2, 4-D (Table 1). The G.P. media was tried with varying levels of sucrose also (Table 2).

The cultures were incubated in dark at 25 ± 2°C. Observations were recorded after 30 days of incubation. for the induction and weight of callus.

Callus induction from immature tassels : Immature tassels were collected from field grown plants of AMC-1. Fresh immature tassels (1 to 5 cm long) enclosed in leaves were isolated from 35 days old plants by removing the exposed leaf sheaths and shoots until 7-10 cm stem section remained. They were surface sterilized with 0.1% $HgCl_2$ for 7 minutes followed by washing in sterile distilled water. Then remaining young leaves were peeled. The tassels were then cut in to 2-3 mm sections and incubated at 25 ± 2° C under continuous low light (1000 lux) on MS medium supplemented with different levels of 2, 4-D as given in Table 3. Observations on callus induction and its weight were recorded after 30 days in incubation.

For differentiation. callus cultures were transferred to G. P. and M. S. basal media supplemented with different auxins (2, 4-D) & IAA) and various concentrations of KIN (Table 4). Cultures were incubated at 25 ± 2°C with a photoperiod of 16 hours. Plantlets developed from callus were transferred from test tubes to polybags for hardening by using different treatments (Table 5).

Table 1. Effect of 2.4-D on the induction of callus from young leaves in AMC-1

Treatments (mg/l)	Average weight of callus (g)**
GP + 2, 4-D (1)	-
GP+ 2, 4-D (2)	1.84 ± 0.25
GP+ 2, 4-D (3)	1.09 ± 0.19
GP+ 2, 4-D (4)	-
GP+ 2, 4-D (5)	-
MS+ 2, 4-D (1)	-
MS+ 2, 4-D (2)	1.30 ± 0.28
MS+ 2, 4-D (3)	0.96 ± 0.15
MS+ 2, 4-D (4)	-
MS+ 2, 4-D (5)	-
N6 + 2, 4-D (2)	0.99 ± 0.18
N6 + 2, 4-D (3)	0.73 ± 0.14

Explants taken from 20 days old seedlings.
** Observations recorded after 30 days of incubation.

Table 2. Effect of different levels of sucrose on the induction of callus from young leaves in AMC-1

Medium (GP+2, 4-D 2mg/l +)	Average weight of callus (g)**
Sucrose 1%	0.94 ± 0.11
Sucrose 2%	1.84 ± 0.25
Sucrose 3%	1.85 ± 0.22
Sucrose 4%	1.92 ± 0.25
Sucrose 5%	1.16 ± 0.17

Explants taken from 20 days old seedlings.
** Observations recorded after 30 days of incubation.

Table 3. Effect of different levels of 2, 4-D on the induction of callus from immature tassel segments*

MS + (mg/l)	Callus induction
2, 4-D (1)	-
2, 4-D (2)	++
2, 4-D (3)	+
2, 4-D (4)	-
2, 4-D (5)	-
2, 4-D (6)	-

* Observations recorded after 30 days of induction
\- No callusing
\+ Poor callusing
++ Profuse callusing

Table 4. Effect of different treatments on the development of plantlets from young leaves and immature tassels derived calli*.

Media used (mg/l)	Average number of Plantlets developed from	
	Young leaves	Immature tassels
MS basal medium	4.2 ± 0.42	4.8 ± 0.47
MS + 2,4-D (0.25)	-	-
MS + KIN (0.5) + IAA (0.5)	-	-
MS + KIN(1) + IAA (0.5)	-	-
MS + KIN(1) + IAA(1)	3.6 ± 0.50	5.7 ± 0.52
GP basal medium	4.4 ± 0.48	5.3 ± 0.40
GP + 2, 4-D (0.25)	-	-
GP + KIN (0.5) + IAA (0.5)	-	-
GP + KIN (1.0) + IAA (0.5)	-	-
GP + KIN (1.0) + IAA (1.0)	3.8 ± 0.49	6.3 ± 0.52

* Observations recorded after 30 days of incubation.

Table 5. Effect of hardening treatments on the survival of plantlets regenerated from leaf and tassel callus.

	Hardening treatments	Survival % plantlets from	
		leaf callus	Tassel callus
M_1S_1	Transfer of plants directly into trays and keeping trays in open.	-	-
M_1S_2	Transfer of plants directly into trays and keeping trays in open.	10	40
M_2S_2	Transfer of plants in sterile distilled water for 6 h and then transferred to trays and keeping the trays in mist	20	50
M_2S_3	Transfer of plants in sterile distilled water for 6 h and then transferred to trays and covering the plants with polythene bags and keeping in open.	-	

Results and Discussion

The study revealed (Table 1) the best callus response when 2, 4-D 2 mg/l was used in different media. It resulted into maximum callusing of 1.84 gm recorded in G.P. medium while in MS medium, the maximum callusing of 1.30 gm was recorded and in N6 medium it was 0.990 gm. Thus, it appeared that 2, 4-D 2 mg/l was best for callus induction as also reported earlier [8,9].

The results indicated (Table 2) callus induction to the maximum extent of 1.92 gm in the treatment GP + 2, 4-D 2 mg/l + sucrose 4% followed by 1.85 gm in 3% sucrose. The callus induction increased with increasing levels of sucrose upto 4%.

Response of tassel explants for callus induction showed that when MS basal media was supplemented with different levels of 2, 4-D, profused callusing was obtained with the treatment MS + 2, 4-D 2mg/l (Table 3). No callusing was obtained in other treatmetns.

The results on callus regeneration (Table 4) revealed maximum plantlet regeneration from leaf callus (4.4) obtained in G.P. basal medium followed by MS basal medium. Where as from tassel callus maximum plantlet regeneration (6.3) was obtained in GP + KIN 1 mg/l + IAA 1mg/l, followed by MS + KIN 1 mg/l + IAA 1 mg/l. These observations confirm the findings of Chang[10] and Conger *et al.*[11], supporting callus differentiation from young leaves in maize and that of Suprasanna *et al.*[12] Songstad *et al*[13] for callus regeneration from tassels.

In *in vitro* multiplication, the cultures are grown in conditions where nutritional and physical factors are so manipulated that the plantlets have the most conducive environment for the growth. In order to have proper establishment and survival of plants so developed in controlled environment, the plantlets need to be hardened before their exposure to outside conditions so that they acclimatize well to the filed conditions.

In the present investigation (Table 5). plantlets survived only in treatment M_1 S_2 (Transfer of plants directly in to trays having pot mix and keeping trays in mist) and M_2 S_2 (Transfer of plants in sterile distilled water for 6 hours and then transferred to trays and keeping the trays in mist). In plantlets developed from tassel callus, the treatment M_2 S_2 resulted in 50 per cent survival and the treatment M_2 S_1 resulted in 40 percent survival, while in case of plantlets developed from leaf callus, maximum survival of 20 per cent was recorded in treatment M_2 S_2 and 10 percent in treatment M_1 S_2. It indicated that other hardening treatments tried in the study were not that much suitable for the survival of the plantlets obtained *in vitro* in AMC-1 variety of maize.

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