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Somatic embryogenesis from three commercially important inbreds of *Zea mays*

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ABSTRACT

Zea mays (maize) genotypes B73, Mol7 and LH38 were evaluated for their capacity to undergo somatic embryogenesis. Over 1500 immature embryos (ie's) of B73, 2900 ie's of LH38 and 400 ie's of Mol7 were excised 10-17 days after pollination and plated on six different media. Overall response, reported as a percentage of the ie's plated that developed embryogenic callus, was 2.1%, 1.6% and 26% for LH38, B73 and Mol7, respectively. Best response on a given medium for each of these genotypes was 9.2% (LH38), 4.4% (B73) and 100% (Mol7). Other parameters examined for their effects on production of embryogenic callus included self vs. sib pollination, ear ranking (1st, 2nd or 3rd ear), and temperature shock, all of which had no significant effect. Plantlets regenerated from selected treatments of B73 have been grown to maturity, selfed or sibbed and seed collected for field evaluation.

ABBREVIATIONS

i.e., immature embryo; 2,4-D, 2,4-dichlorophenoxyacetic acid;

INTRODUCTION

The regeneration of maize from tissue culture has been documented for a decade (Green & Phillips, 1975) and regeneration via somatic embryogenesis has been documented for three years (Green, 1982; Armstrong & Green, 1982; Lu et al., 1982; Vasil et al., 1983; Novak et al., 1983). Most maize regeneration systems described to date have dealt with hybrid germplasm, the inbred A188 or other inbreds of limited commercial value. The manipulation of commercially important corn belt inbreds may, however, provide the greatest opportunity for the creation of unique elite germplasm.

Initial studies performed in the summer of 1983 surveyed the regenerability, via either embryogenesis or organogenesis, of 24 inbred and 14 hybrid maize genotypes on up to 15 different media. Four of these media and three of the inbred genotypes which are of commercial importance (B73, Mol7 and LH38) were then selected for a more rigorous evaluation of regeneration via somatic embryogenesis.

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MATERIALS AND METHODS

DONOR PLANTS

All B73 and LH38 plants were grown through anthesis in a greenhouse with auxiliary, high intensity discharge lighting. Some of the Mol7 plants were placed outdoors in the period July-August, 1984 (Syracuse, NY), which improved the synchrony of pollen shed and silking. Plants were either self or sib pollinated according to standard procedures.

PLATING PROCEDURE

Ears were removed from plants at 10-17 days post-pollination and surface sterilized within 30 minutes of removal by agitation for 20 minutes in 50% Clorox with 1% Fisher Versa-Clean laboratory detergent. Plating was done essentially as described by Green & Rhodes (1982): Kernel crowns were excised and ie's were aseptically removed. They were placed, scutellum side up, on semi-solid medium at a density of 7 per 100 x 25 mm plate (20-25 ml medium volume), and incubated at 28°C in the dark. Fourteen days after plating, zygotic embryos which had precociously germinated were removed, and scutellar callus was excised away from the embryo axis and subcultured. This material was then incubated under a 16:8 LD photoperiod (40μE $m^{-2}s^{-1}$) at 28°C. Callus was subcultured to fresh medium every three weeks and transferred to regeneration medium (without 2,4-D) as somatic embryos appeared. Cultures were initiated from 2900 explants for LH38, 1500 for B73 and 400 for Mol7.

MEDIA

Media were adapted from previously published formulations and are presented in Table 1. The first two media (N6Pro and MSPro) were adapted from those used by Armstrong & Green (1982). The inclusion of 25 mM L-proline in these media is of special interest since it has been shown (Armstrong & Green, 1982) that it could dramatically stimulate embryogenesis from the genotype A188. The third medium selected (RMM6) was modified from a maize suspension culture formulation described by

Polikarpochkina et al. (1979). This medium contains 6 mg/l 2,4-D and high (1 g/l) casein hydrolyzate. The fourth medium (PHL) selected was adapted from a liquid medium developed by Potrykus et al. (1979). This medium is characterized by a high osmoticum (36.4 g/l mannitol plus 20 g/l sucrose) and contains 10% coconut water. The fifth medium utilized was PHL with the addition of 25 mM L-proline (PHL+Pro). The final medium used is a modification of the Yu-pei anther culture medium (Rapela, 1984), significant for its high osmotic potential and the presence of 3.5 mM L-proline.

TEMPERATURE SHOCK

Temperature shock treatments were conducted with ie's of BMS x A188, LH38 and A188. Temperature treatments included a 1 or 2 hr treatment at 40°C (performed by floating Parafilm-sealed plates in a 40°C water bath), an 18 hr treatment at 3-5°C and a control group kept at 28°C in the dark. Temperature shock treatments were performed immediately after ie's were plated.

TABLE 1. COMPARISON OF CULTURE MEDIA

	YP-Rap	RMM6	N6Pro	MSPro	PHL	PHL+Pro
MACRONUTRIENTS(mM)						
KNO_3	25	30	28	18.8	12.0	12.0
NH_4NO_3	2.1	-	-	20.6	8.0	8.0
$CaCl_2.2H_2O$	1.2	1.0	1.2	3.0	4.0	4.0
KH_2PO_4	2.9	3.4	2.3	1.25	1.0	1.0
$MgSO_4.7H_2O$	1.5	1.8	0.77	1.5	1.0	1.0
$(NH_4)_2SO_4$	-	1.1	3.5	-	-	-
MICRONUTRIENTS(uM)						
$MnSO_4.H_2O$	20	59	20	99.4	50	50
$ZnSO_4.7H_2O$	5.2	6.9	5.2	36.9	20	20
H_3BO_3	26	49	26	100	50	50
KI	4.8	4.5	4.8	5.0	5.0	5.0
$NaMoO_4.2H_2O$	-	1.0	-	1.1	1.1	1.1
$CuSO_4.5H_2O$	-	0.01	-	0.1	0.1	0.1
$CoCl_2.6H_2O$	-	0.01	-	0.1	0.1	0.1
$FeSO_4.7H_2O$ (as Fe-EDTA)	100	100	100	100	100	100
ORGANIC AMENDMENTS (mg/l)						
glycine	7.7	-	2.0	2.0	2.0	2.0
L-proline	400	-	2880	2880	-	2880
casein hydrolysate	500	1000	300	-	-	-
myo-inositol	-	80	-	100	90	90
thiamine.HCl	0.25	0.4	0.1	0.1	8.5	8.5
pyridoxine.HCl	0.25	0.1	0.5	-	1.0	1.0
nicotinic acid	1.3	-	0.5	1.0	6.0	6.0
Ca.pantothenate	0.25	-	-	-	-	-
2,4-D	1.0	6.0	1.0	1.0	2.0	2.0
OTHER ORGANICS (g/l)						
sucrose	120	20	30	30	20	20
mannitol	-	-	-	-	36.4	36.4
coconut water	-	-	-	-	100	100
agar (Difco, Noble)	6.0	6.0	6.0	6.0	6.0	6.0

RESULTS AND DISCUSSION

Crossing a highly regenerable inbred (A188) with a non-regenerable, commercially valuable inbred could eventually lead to development of a regenerable, elite inbred after sufficient backcrossing and selection. Preliminary studies were conducted in the summer of 1983 with 24 inbreds and 14 hybrid crosses (primarily to A188). These studies employed 15 different culture media spanning a range of plant growth regulators, carbon sources, osmotically active substances, amino acids and nitrogen sources and concentrations. The number of plants ultimately regenerated was too low to allow any specific conclusions to be drawn from the preliminary study, however, this study resulted in the identification of five media that were effective across a wide range of genotypes (Table 2). Additionally it confirmed the observations reported in the literature for many species that with a given set of media and environment conditions, regenerability is extremely genotype dependent. Four of these media were selected for further evaluation in the summer of 1984 and two additional media were added to further examine the effects on embryogenesis of L-proline and a high sucrose medium originally developed for maize anther culture (see Table 1). Three of the inbred genotypes which are of commercial importance (B73, Mol7 and LH38) were then selected for a more rigorous evaluation of their ability to produce embryogenic cultures.

TABLE 2. PERFORMANCE OF MAIZE GENOTYPES ON SELECTED MEDIA

MEDIUM	# OF GENOTYPES REGENERATED[a]
N6Pro[b]	15
MSPro[b]	15
PHL[b]	13
RUP[b,c]	10
RMM[b,d]	6
SHD[e]	2
N6M[f]	0
MS-P[g]	0
MS11[g]	0
MSZM[g]	0
MSDKG[g]	0

[a]Thirty eight genotypes were examined, including B73, Mol7, LH38, LH74, W64A, WF9, T232 and SD10, either as inbreds or in crosses with A188.

[b]Selected for further evaluation

[c]From Jones et al. (1981)

[d]From Polikarpochkina et al. (1979)

[e]From Conger et al. (1983)

[f]Adapted from Chu (1981) to include 25 mg/l L-proline and 100 mg/l casein hydrolysate

[g]Adapted from Murishige and Skoog (1962) by the removal of all plant growth regulators (MS-P), the addition of 1 mg/l ea. of 2,4-D and kinetin (MS11), 3 mg/l 2,4-D and 0.3 mg/l kinetin (MSZM), or 3 mg/l 2,4-D, 0.3 mg/l kinetin and 1 mg/l Gibberillic acid GA_3 (MSDKG).

Overall response of the almost 5000 ie's plated is summarized in Table 3. Nearly 100% of the ie's plated produced callus. The percentage of explants which produced embryogenic callus was 2.1% for LH38, 1.6% for B73 and 26% for Mol7. The PHL medium (which has a high osmotic potential), was clearly the most effective medium for inducing embryogenesis in LH38 and B73. In contrast, the media developed by Armstrong & Green (1982) [MSPro and N6Pro], for A188 was only marginally effective for the three genotypes. It is of particular interest that addition of 25 mM proline to the most effective medium (PHL) eliminated the embryogenic response for both LH38 and Mol7 and reduced by half the response with B73 (from 4.4% to 1.9% of the explants responding). Other media induced little or no embryogenesis for these two genotypes except YP-Rap, which induced a moderate response for B73 (2.7%). Mol7 responded dramatically (100%) to YP-Rap, which has a high osmotic potential and contains 3.5 mM proline. Also, PHL induced greater embryogenesis from A188 than N6Pro (Table 5 and data not shown). The addition of 25 mM proline to PHL did not have a large negative effect on rates of embryogenesis from A188, contrary to what might be expected based on the data for B73, Mol7 and LH38.

TABLE 3. SOMATIC EMBRYOGENESIS FROM MAIZE INBREDS

	LH38	B73	Mol7
Ie's plated	2903	1566	442
Embryogenic ie's	59	24	108
Plantlets	2	28	0
Response:[a]			
PHL	9.2% (50/544)	4.4% (15/340)	5% (3/55)
RMM6	0 (0/559)	0 (0/286)	0 (0/40)
MSPro	1.1% (6/538)	0 (0/270)	0 (0/40)
N6Pro	0 (0/529)	0.4% (1/282)	2% (2/114)
PHL+Pro	0.3% (1/370)	1.9% (4/216)	0 (0/62)
YP-Rap	0.8% (2/250)	2.7% (4/149)	100% (103/103)
Overall Response	2.1% (59/2790)	1.6% (24/1543)	26% (108/414)

[a]Embryogenic calli/explants plated

There was no significant effect of ear position (ie's from 40 primary, 9 secondary, 4 tertiary and 1 quaternary ear were plated) on somatic embryogenesis medium (Table 5). Neither were there any significant effects of self vs. sib pollination (ie's from ears of 28 of the former and 26 of the latter were plated).

TABLE 4. POLLINATION AND EAR POSITION EFFECTS ON EMBRYOGENESIS

	LH38	B73	Mo17
Ears plated:	26	20	8
Responding ears/ Total ears:	69% (18/26)	40% (8/20)	100% (8/8)
Selfs	70% (14/20)	50% (3/6)	100% (2/2)
Sibs	67% (4/6)	36% (5/14)	100% (6/6)
Primary ears	60% (12/20)	31% (4/13)	100% (7/7)
Secondary ears	100% (6/6)	50% (1/2)	100% (1/1)
Tertiary ears	-	75% (3/4)	-
Quaternary ears	-	0% (0/1)	-

The anther culture literature indicates that temperature shock (either heat or cold) can have a dramatic effect upon androgenesis. Initial observations of the effect of heat shock (2h@40°C) on freshly excised embryos of A188, BMS x A188 and SD10 x A188 indicated that there may be a beneficial effect (data not presented). Further experiments, however, demonstrated a lack of positive response to temperature shock (Table 5). When responses were pooled across media for A188, there was no increase in the induction of embryogenesis by any of the heat pre-treatments and there was a reduction in embryogenic frequency caused by the cold pretreatment. For LH38, although the number of explants used may have been too low to be able to detect negative effects, neither heat nor cold treatment produced any significant increase in response compared to controls.

TABLE 5. RESPONSE OF A188 AND LH38 TO TEMPERATURE SHOCK[a]

		TEMPERATURE TREATMENT			
G'type	Medium	control 28°C	1h@ 40°C	2h@ 40°C	18h@ 3°C
A188	PHL	6/7	6/7	3/4	4/7
A188	PHL+Pro	5/7	4/7	4/7	2/7
A188	YP-Rap	0/5	0/7	2/7	1/7
A188	N6Pro	4/7	3/7	3/7	1/7
Total		15/26 (58%)	13/28 (46%)	12/25 (48%)	8/28 (29%)
LH38	PHL	2/21	1/14	1/14	0/14
LH38	PHL+Pro	0/21	0/14	0/14	0/14
LH38	MSPro	0/14	0/14	0/14	0/14
LH38	N6Pro	0/14	0/14	0/14	0/14
LH38	RMM6	0/21	0/14	0/14	0/14
Total		2/91 (2.2%)	1/70 (1.4%)	1/70 (1.4%)	0/70 (0.0%)

[a]Indicated as embryogenic calli/explants plated

R_o plantlets from embryogenic cultures of B73 on PHL medium were grown to maturity and either selfed or backcrossed to seed-derived B73. Fifty-six R_1 plants were then grown in the greenhouse, selfed, sibbed or backcrossed and the seed (R_2) of 50 of them is being evaluated for genetic stability in field trials.

It should therefore be possible to regenerate other genotypes of commercial importance, via somatic embryogenesis, without resorting to approaches such as breeding for regenerability (e.g. by crossing to A188). The ability to induce somatic embryogenesis from heretofore recalcitrant inbred genotypes represents an opportunity for the application of other, currently available tissue culture techniques such as somaclonal variation to traditionally based corn breeding programs.

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