MAIZE (ZEA MAYS L.) IMPROVEMENT STUDIES

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Abstract. *Plant regeneration via tissue culture is becoming increasingly more common in monocots such as maize (Zea mays L.). Pollen (gametophytic) selection for resistance to aflatoxin in maize can greatly facilitate recurrent selection and the screening of germplasm for resistance at much less cost and in a shorter time than field testing. In vivo and in vitro techniques have been integrated in maize breeding programmes to obtain desirable agronomic attributes, and enhance the genes responsible for them and speed up the breeding process. The efficiency of anther and tissue cultures in maize and wheat has reached the stage where they can be used in breeding programmes to some extent and many new cultivars produced by genetic manipulation have now reached the market.*

Keywords*:* *Zea mays L.*, callus induction, aflatoxin, grain yield

1. Introduction

The regeneration of plants from tissue cultures was first reported in maize (*Zea mays L.*) by Green and Philips [6], who utilised immature embryos as the tissue source. Using the same tissue system, Springer et al. [19] demonstrated that plant regeneration took place by means of organogenesis.

Rice et al. [17] found that plant regeneration could also occur by somatic embryogenesis. Both types of regeneration arise from hard, white or yellow callus, which can be clearly distinguished from the granular, greyish-yellow, translucent callus that is incapable of plant regeneration. The regeneration of maize from cell and tissue cultures has been limited to a few specific genotype and medium combinations (Green and Philips, [6]; Hodges et al., [7]; Kamo et al., [8]. Medium improvements boosted the average regeneration, but genotypic differences remained (Duncan et al.,) [3]. Studies on the genotype component can be used to predict probabilities for a desired response level and to describe in more detail the nature of the tissue culture response (Tomes and Smith) [21].

Mycotoxins, in general, reportedly contaminate about one-quarter of the world's yearly food and feed crops. The effects of mycotoxin consumption on animal health range from decreased growth rates and reproductive efficiency to mortality. In addition, there is increasing concern about the effects of aflatoxin on human health, as aflatoxins have been linked to liver cancer. The occurrence of aflatoxin in food is viewed as a potential threat to the food supply. It has been deemed

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necessary to develop efficient methods that will prevent aflatoxin contamination in crops. Host-plant resistance is the most logical and useful method of control (Dickson et al. [2], Kang et al., [5]).

Thus far, only a few studies on the inheritance of resistance have been conducted (Widstrom et al., [23]; Gardner et al., [4]; Darrah et al., [1]; Gorman and Kang, [9]; Pepó and Szabó, [16]; Tóth and Bódi, [22]). Progress in elucidating genetic mechanisms and identifying sources of resistance to aflatoxin has been slow, primarily because genotype evaluation in the field (sporophytic selection) is laborious, expensive and time-consuming. Selection at the pollen level (male gametophytic selection) to screen for resistance to aflatoxin did not receive much attention until recently. The gamatophytic generation has appropriately been called 'the forgotten generation'.

A total of 60-70 % of structural genes controlling traits in the sporophytic generation (plant) are expressed in the gametophytic generation (pollen) (Mulcahy, [11]; Mulcahy and Mulcahy, [12]; Ottaviano et al., [14]; Tanksley et al., [20]; Smith, [18]). This genetic overlap between the sporophytic and gametophytic generations offers a tremendous potential for modifying the sporophyte by applying selection pressure on the gametophyte. A maize plant produces 2 to 5 million pollen grains that can be subjected to selection. Selection pressure applied to pollen produced by a genetically homogeneous, heterozygous plant is expected to produce genetic changes in the sporophytic population (Ottaviano and Mulcahy, [14]).

Intergenic somatic hybridization was performed between albino maize *(Zea mays L.)* protoplasts and mesophyll protoplasts of wheat (Triticum aestivum L.) by means of PEG treatments. None of the parental protoplasts were able to produce green plants without fusion (Mórocz et al., [10]).

2. Materials and methods

Two maize populations developed in the Louisiana State University maize breeding program, viz. (Mo17 x B73) x Yellow Creole and (Mo17 x B 73) x (L331 x Yellow Creole), hereafter referred to as population L91 R and population L331, respectively, were evaluated for their in vitro culturability and regeneration potential. The S0, S1 and S2 generations were subjected to tissue culturing using the following procedure. The seeds were surface-sterilised for 10 min in 0.2% aqueous mercurous chloride solution, rinsed overnight under running tap water and re-sterilised for 5 min in 0.2% aqueous mercurous chloride solution, followed by several water rinses. Twenty-five kernels of each generation were germinated. Aseptic seedlings were grown on a 1% agar-solidified medium cotaining the inorganic constituents of Murashige and Skoog [13]: 3% sucrose, 26.7 µM glycine, 4.1 µM nicotinic acid, 2.4 µM pyridoxine-HCl and 0.3 µM thiamine-HCl.

N6 medium was used with 100 µmol Fe-EDTA concentration to initiate anther culture. Saccharose concentration 10% supplemented with activated charcoal was applied to induce calli in maize.

The radicles were aseptically separated from the plumules at the scutellar node and the explants were cut into five pieces each 2̃3 mm long. These explants and intact, mature embryos were plated on MS medium. The pH was adjusted to 5.8 before autoclaving. Incubation was done at 26 °C with a 16/8 h photoperiod.

The 2,4-D concentration was 2.5 mg/L-1. After callus induction, meristematic segments were discarded; the remainder were transferred to the above culture medium for callus proliferation. To induce further differentiation, the calli were subcultured on MS medium supplemented with different concentrations of 2,4-D and zeatin. Regenerated plantlets were transferred to hormone-free medium for root development.

Anthers with mid-uninucleate microspores were cold-treated at 8-10 °C for 14 days and heat-preincubated in the dark at 27± 2 °C for 7 days. To increase the frequency of embryoids the medium was supplemented with activated charcoal and 0.1 mg L-1 TIBA. The embryoids were further cultured on N6 and M9 media to induce differentiation.

In the breeding programme, diallels were obtained by crossing four maize inbred lines (P2, P34, P50 and P61). The general and specific combining abilities for callus weight and grain yield were estimated using a full diallel system according to a modified version of Griffing's 1 method.

3. Results

It can be seen from Table 1 that the callus induction frequency for the two populations ranged from 4.7 to 60.9%. The highest frequency of callus formation was exhibited by radicle tissue and the lowest by the embryo. In both populations, the callus induction frequency decreased as the level of homozygosity increased, which suggested that callus induction was controlled primarily by dominant gene action.

Table 1) Callus induction and regeneration for populations L331 and L91R

Explant	Inbred stage	Callus induction		Regeneration	
		L331	L91R	L331	L91R
Radicle	S_o	60.9	41.1	4.75	1.51
	S_1	58.0	32.0	4.45	0.0
	S_2	56.0	23.9	0.0	0.0
	Mean	58.3	33.4	2.70	0.59
Plumule (P)	S_o	41.9	30.1	9.94	3.05
	S_1	39.0	23.9	6.65	1.26
	S_2	38.9	19.0	4.43	0.0
	Mean	40.0	25.1	6.95	1.65
Embryo (E)	S_o	40.0	9.8	5.00	2.17
	S_1	38.2	7.2	2.06	1.20
	S_2	35.8	4.7	1.05	0.0
	Mean	38.0	7.5	2.75	1.25
Mean: R+P+E	S_o	50.9	33.3	7.03	2.26
		(601/1181)	(228/1016)	(83/1181)	(23/1016)
	S_1	47.9	26.1	4.79	0.67
		(590/1232)	(233/893)	(59/1232)	(6/893)
	S_2	46.9	19.9	3.92	0.0
		(577/1230)	(134/673)	(25/1230)	(0/673)

The explants had different plant regeneration percentages in both populations. The maximum number of plants was regenerated from plumules in the L331 population. No plant regeneration was noted in the S2 generation of the L91R population. The results suggested that plant regeneration would be increasingly more difficult in the inbred generations and that prior to embarking on a tissue culture-based breeding programme, responsive genotypes should be identified.

Microscopic investigations were carried out to investigate the effect of Aspergillus flavus spores and the aflatoxin B1 on in vitro pollen germination and it was demonstrated that both inhibited pollen germination (Table 2).

Table 2) Mean percentage germination of pollen grains from the F_2 of the single cross (Mp 313 E *x* SC 212 M)

Treatment	Mean pollen grain germination (%)
Control	71.5 a*
Aspergillus parasiticus spores	44.2 b
Aflatoxin B1 (400 ppm)	42.4 b
Aspergillus flavus spores	23.7 c

- Means followed by the same letter are not significantly different at the 5% level of probability according to Duncan's New Multiple Range Test.

The callus induction and yielding ability of four maize inbred lines (P2, P34, P50 and P61) were compared in a diallel system. The general and specific combining

abilities for callus weight and grain yield were estimated (Tables 3, 4, 5 and 6) using a full diallel system according to a modified version of Griffing's 1 method.

Table 3) Values of general combining ability (GCA) for callus weight

Inbred lines	GCA [g]
P 2	0.1110
P 34	-0.0840
P 50	-0.7545
*P 61	0.7180

* Registered maize line

Table 4) Values of general combining ability (GCA) for grain yield

Inbred lines	GCA [t/ha]
P 2	-0.384
P 34	-0.093
P 50	-0.073
*P 61 (3)	0.550

* Registered maize line

Table 5) Results of callus induction in diallel analysis (SCA = specific combining ability (g), RE = Effect of reciprocal (g))

Female lines	Male lines			
	P 2	P 34	P 50	P61
P 2	-4.438 (SCA)	1.858 (SCA)	1.719 (SCA)	0.861 (SCA)
P 34	-2.250 (RE)	-2.988 (SCA)	0.024 (SCA)	1.106 (SCA)
P 50	-2.080 (RE)	-0.500 (RE)	-5.825 (SCA)	4.083 (SCA)
P 61	-1.995 (RE)	-0.955 (RE)	-0.720 (RE)	-6.050 (SCA)

(SCA = specific combining ability (g), RE = Effect of reciprocal (g))

*$LSD_{5\%}$ = 0,237

R^2 = 0,898

Table 6 Results of grain yield in diallel analysis

Female lines	Male lines.			
	P 2	P 34	P 50	P61
P 2	-5.333 (SCA)	2.406 (SCA)	1.137 (SCA)	1.789 (SCA)
P 34	-0.455 (RE)	-4.436 (SCA)	0.657 (SCA)	1.373 (SCA)
P 50	-1.108 (RE)	0.109 (RE)	-4.059 (SCA)	2.265 (SCA)
P 61	-1.114 (RE)	-0.260 (RE)	-1.361 (RE)	-5.426 (SCA)

(SCA = specific combining ability (t/ha), RE = Effect of reciprocal (t/ha)

*$LSD_{5\%}$ (3) = 0,312 R^2 = 0,887

3. Conclusions

A wide variety of diploid and double haploid lines produced in vitro are increasingly used in practical breeding programme.

Anther culture and tissue culture techniques were employed to develop lines with resistance to aflatoxins and herbicides, and with other desirable agronomic traits such as fast dry-down rate, better stalk and seed quality, and weevil resistance. As seen in Figure 2, a complex maize breeding programme was developed to obtain desirable agronomic attributes, enhance the genes responsible for them and speed up the breeding process. Depending on the nature of the source material, e.g. synthetics, F1 or open-pollinated varieties, and the breeding aims, one or more haploid (pollen) or diploid (tissue) steps were included and the F1 hybrids or selfed progenies in later generations served as the source material for haploidization or tissue culture. One haploid step followed by selection in the greenhouse/field or a diploid step after selection at the cell/plant level during the first androgenetic generations (A1) or diploid progenies (D1) and two subsequent selfed generations (A2, A3, D2, D3) proved to be the most efficient procedure, if characters from related varieties were to be combined. To make this genetic manipulation system more efficient, it was combined it with several backcrosses.

A combination of conventional and new genetic recombination methods (in vivo and in vitro genetic manipulation) may result in the development of cereal varieties and hybrids that are better able to meet production demands. The efficiency of anther and tissue cultures in most cereals such as maize has reached the stage where it can be used in breeding programmes to some extent and many new cultivars produced using this system have now reached the market.

REFERENCES

[1] Darrah, L. L., Lillehoj, E.B., Zuber, M. S., Scott, G. E., Thompson, D., West, D. R., Widstrom, N. W., Fortnum B,. A.,: *Inheritance of aflatoxin B1 levels in maize kernels under modified natural inoculation with Aspergillus flavus.* Crop Sci. 27, 869-872. (1987)

[2] Dickson, J. I., Dronovalli, S., Pepo, P., Kondapi, N., Kang, M.S.: *Effect of MSMA on in vitro corn pollen germination.* Southern Branch of American Societiy of Agronomy, Lexingston, Kentucky. Agronomy Abstracts, 45-46. (1992)

[3] Duncan, D. R., Williams, E. M., Zehr, B. E., Widholm. J. M.: *The production of callus capable of plant regeneration from immature embryos of numerous Zea mays genotypes*. Planta 165, 322-332. (1985)

[4] Gardner, C.A. C., Darrah, L. L., Zuber, M. S., Wallin, J.R.: *Genetic control of aflatoxin production in maize*. Plant Dis. 71, 426-429. (1987)

[5] Kang. M.S.: *Preharvest aflatoxin contamination in maize: Resistance and genetics*. Plant Breeding, 107, 1-10. (1991)

[6] D Green, C. E., Philips, R. L.: *Plant regeneration from tissue culture of maize.* Crop. Sci., 15, 417-421. (1975)

[7] Hodges, T. K., K.K. Kamo, R. M. Becwar, S. Schroll.: *Regeneration of maize.* In: Biotechnology in Plant Science: *Relevance to Agriculture in the Nineteen Eighties*. Zaitlin, M., Day, P., Hollaender, A. (eds.), Academic Press, Orlando, Florida: pp.15-33.P. (1985)

[8] Kamo, K. K., Becwar M. R., Hodges, T. K.: *Regeneration of Zea mays L. from embryogenic callus.* Bot. Gaz. 146, 327-334. (1985)

[9] Gorman, D. P.Kang, M.S., Pepó, P., Dronovalli, S., Dickson, J. I., Kondapi, .N.: *Corn breeding and genetic investigations,* Report of Projects for 1991., Dept. of Agronomy, LSU. 71-78. (1992)

[10] Mórocz S., Dudits D., Golovkin M. V., Ábrahám M., Bottka S., Fehér A.*: Production of transgenic maize plants by direct DNA uptake into embryogenic protoplasts.* Plant Science, 90, 41-52. (1993)

[11] Mulcahy, D. L.: *Correlation between gametophytic and sporophytic characteristics in Zea mays L.* Science, 171, 1155-1156. (1971)

[12] Mulcahy, D. L., Mulcahy., G. B.: *The effects of pollen competition.* Am. Scientist 75, 44-50. (1987)

[13] Murashige T., Skoog F.,: *A revised media for rapid growth and bioassays with tobacco tissue culture*. Physiol Plant 15, 473-479. (1962)

[14] Ottaviano, E., Sari-Gorla, M.,Mulcahy D. L.: *Pollen tube growth rates in Zea mays: Implications for genetic improvement of crops.* Science 210, 437-438. (1980)

[15] Ottaviano, E., Mulcahy, D. L.: *Genetics of angiosperm pollen.* In: Scandalios, J. G. (ed.) Advances in Genetics, Academic Pres, Inc., CA. pp.1-64. (1989)

[16] Pepó, P., E. Szabó.: *RAPD markerek öröklődése kukoricában (Zea mays L.) (Inheritance of RAPD markers in maize (Zea mays L.)).* IV. Növ.nem. Tudományos Napok, MTA, Abstract, 122. (1998)

[17] Rice, T. B., R. K. Reid, P. N. Gordon.: *Morphogenesis in field crops.* pp. 262-277. Hughes, K. W., Henke, R., Constantin, M. (eds.), *Propagation of Higher Plants through Tissue Culture.* National Technical Information Service, U. S. Department of Commerce, Springfield, VA., USA. (1978)

[18] Smith, G. A.: *Sporophytic screening and gametophytic verification of phytotoxin tolerance in sugarbeet (Beta vulgaris L.).* pp. 83-88. In Mulcahy, D. L., Mulcahy, G. B. and Ottaviano, E. (eds.) Biotechnology and Ecology of Pollen Springer-Verlag, New York. (1986)

[19] Springer, W. D., Green, E. C., Kohn, A. K.: *A histological examination of tissue culture initiation from immature embryos of maize.* Protoplasma 101, 269-281. (1979)

[20] Tanksley, S. D., Zamir, D., Rick, C. M.: *Evidence for extensive overlap of sporophytic and gametophytic gene expression in Lycopersicum esculentum.* Science 213, 453-455. (1981)

[21] Tomes, D. T., Smith, O. S.: *The effect of parental genotype on initiation of embryogenic callus from elite maize (Zea mays L.) germplasm.* Theor. Appl. Gen. 50, 505-509. (1985)

[22] Tóth, Sz., Bódi, Z.: *Indukált mutációval létrehozott kukoricagénbank a nemesítési alapanyagbázis növelésért. (Gene Bank Developed by Induced Mutation for Selection)* Acta Agraria Debreceniensis. 19, 45-49. (2006)

[23] Widstrom, N. W., Wilson, D. M., McMillian, W. W.: *Ear resistance of maize inbreds to field aflatoxin contamination.* Crop Sci. 24, 1155-1157. (1984)