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Results of a diallel trial and a breeding experiment for in vitro aptitude in maize

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Summary. Some characteristics of in vitro culture of somatic tissues of maize were analysed by a diallel trial. Eight genetically different pure strains, chosen for their aptitudes, were used. The results show that there is considerable genetical variation for the characteristics of in vitro culture and that it should be possible to breed for aptitude to in vitro culture. The linear regression of hybrids on mid-parent reveals a significant heritability for such aptitude. Through selection we have improved plant regeneration after a long period of callus growth.

Key words: *Zea mays* L. – In vitro culture – Genetic variance – Diallel trial – Breeding for in vitro aptitude

Introduction

Genetical variations in tissue culture have been widely reported in numerous species for all kinds of tissues: somatic tissue, gametes and endosperm. Genetical variability in maize (*Zea mays* L.) was investigated for such characters as initiation and maintenance of callus growth, in vitro organogenesis (leaves, roots) and regeneration of whole plantlets with a normal development (Beckert and Pollacsek 1979). The regeneration rate (expressed as the ratio of scutella number giving at least one normal plantlet to the total of cultivated scutella) decreases dramatically with the duration of callus growth.

The authors have tried through genetical selection to improve plant production after in vitro tissue culture. In alfalfa Bingham et al. (1975) increased the percentage of regenerating calluses by genetical selection. A more precise study made on red clover (Keyes et al. 1980) indicated that most of the in vitro culture characteristics have a strong additive genetical variance. Analysing a diallel trial in tomato, Frankenberger et al. (1981) demonstrated that additive genetic effects were associated with non-negligible dominance effects. They reported a very high heritability of the shoot forming capacity in tomato through in vitro culture.

In maize, the mean of the genetic variance and the relative parts played by dominance and additivity were investigated in a full diallel trial. This included all the different steps of plant production from the cultivated scutella. The objective was to obtain genotypes with a high regeneration ability after a long period of in vitro callus growth. The aim of the program was to improve this characteristic through genetical selection.

Material and methods

1 Plant material

All plants were grown during the spring in a greenhouse. For Experiment 1 "the diallel trial": eight different pure strains, maintained at Clermont-Ferrand by manual selfing, were chosen according to their known capacity for the in vitro culture procedure (Beckert and Pollacsek 1979) =

Strain A 641	Pedigree ND 203 × B 14
A 188	'Silver King' × 'NW Dent'
Cm 182	WD × B 8
F 1852	FC 32 × F 19
FV 83	'Land race'
F 1243	FC 30 × FC 49
F 1048	F 115 × F 22
MS 1334	'Golden Glow' × 'Maize Amargo'.

For Experiment 2, the breeding experiment: a sample of scutella obtained from the cross between two F_1 hybrids (A 188 × A 641) and (W 751 × B 8) was used.

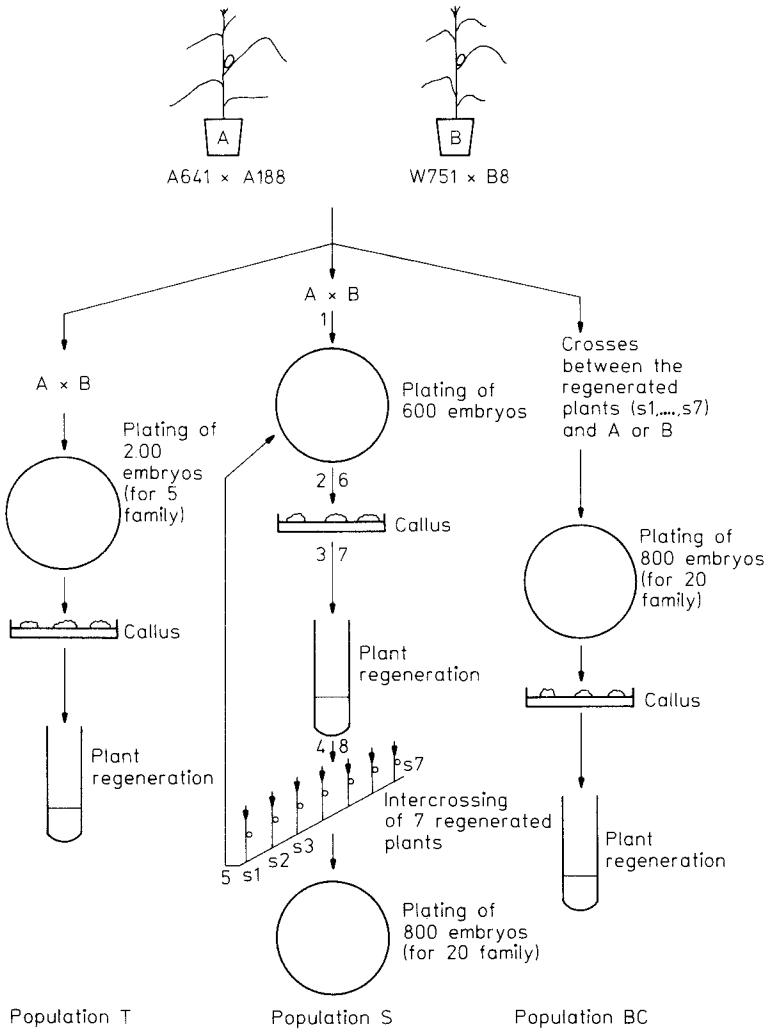

Fig. 1. Scheme for producing maize plants with an improved ability for in vitro culture

2 Mating design

All the crosses of the diallel design were made manually with both selfing and crossing (normal and reciprocal) through pollination of silks measuring about 10 cm length.

The breeding method to improve the ability to the in vitro procedure is described in Fig. 1. The intercrossings between the progenies of the seven chosen regnerated plants were randomly made.

3 In vitro method

The immature embryos were plated when the scutellum length reached about 2 mm (Beckert 1982). Kernel dissection and embryo plating were carried out according to the recommandations of Green and Phillips (1975).

The media and other culture conditions were those described by Beckert and Pollacsek (1979). The media were supplemented with 2 mg/l of Pca (Parachlorophenoxyaceticacid), 2-4-D (2-4 dichlorophenoxyaceticacid) or 2-4-5-T (2-4-5 tricholorophenoxyaceticacid) as auxins.

The cultures were grown under the same conditions for 28 days (Beckert and Pollacsek 1979). After 28 days, a piece of callus, of standard size and developed from a scutellum, was transferred to identical fresh medium. This was repeated every 28 days.

To evaluate their differentiation capacity, pieces of callus were transferred onto medium with lower concentrations of the same auxinic regulator. Plants were then regenerated with transfer to test tubes on the same medium but without growth regulators. Finally, plants with a well-developed root system were planted in soil.

4 Data collection

Before transfer, callus size, the number and vigour of roots or root-like organs, the number and vigour of leaves or leaf-like organs were determined. These notations were made on a scale of 8 (1 for the lowest, 8 for the highest development).

5 Data analysis

For the diallel trial data, analyses were based on the full model of Griffing (1956). The heritabilities in the narrow sense were estimated as the correlation between the mid-parent and their progeny.

For the breeding experiment the analyses of variance were made on the means of each family compounding the three populations T (original population), S (selected material) and BC (offsprings derived from crosses between selected plants and the F_1 hybrids A 641 × A 188 or W 751 × B 8).

6 Repetition number

For the diallel experiment, the data were collected before the 1st, the 2nd and the 3nd transfer. They were collected on 21 embryos plated on medium with P ca (medium P), 21 embryos plated on medium with 2-4-D (medium D), and 21 embryos plated on medium with 2-4-5-T (medium T).

For the breeding experiment the number of plated scutella is given in Fig. 1. Since the purpose was to improve the capacities of scutella cultured for a long time to develop plants, the three populations were compared after the fifth subculture.

Results

Experiment 1: diallel trial

Table 1 presents the main results of the diallel trial. Variances were estimated according to Griffing's model for data collected on the first three passages. Only the values of the Fisher test are reported in this Table. The confidence limits (5%) are given in the last column. For the three characters: shoot formation, root formation, and more especially, callus growth, the intra-genotypical variation appears to be high. This may result from an embryo effect which cannot be disregarded and this could be connected to the variation in physiological state between the embryos. The variation for the three characters is very great between the different dates. This is due to the strong decrease in growth capacity and shoot forming capacity from the first to the third passage; it can also explained by the smaller increase of root forming capacity with length of in vitro culture. The results presented here indicate that for the three characters measured, variation between the different genotypes is always clear but it should be noted that there is an interaction between the varieties and the dates. This suggests that the increase in root forming capacity and the decrease in callus growth or shoot forming capacity with time is not the same for all genotypes compared. Also, there are differences between the media used for the amplitude of these variations but a general rule cannot be made. It seems that it might be easier to compare the shoot forming capacity of different genotypes on medium P or on medium D because the variation is greater than on medium T. For root forming capacity it might be easier to measure on medium T for the same reason.

An evaluation of combining ability was made using Griffing's model. Examining the partition of the genotypic effect it is found that the general combining ability (GCA) is usually high. It appears to be the highest for aptitude to morphogenesis on the media containing Pca as auxins. The specific combining ability (SCA) is also significant but lower, medium P cannot be differenciated from the others. The results show that there are differences between reciprocal

Table 1. Main results of the diallel trial and estimation of the heritabilities

Item	df	Callus growth			Shoot forming capacity			Root forming capacity			Confidence limit (5%)
		Medium P	Medium D	Medium T	Medium P	Medium D	Medium T	Medium P	Medium D	Medium T	
In genotypes	20	34.2	30.8	30.0	0.9	9.6	6.4	8.4	8.9	1.0	1.88
Dates	2	309.6	268.1	562.0	150.5	142.4	144.2	39.2	11.4	16.0	4.60
Genotypes	63	23.0	20.3	22.6	21.0	21.8	14.0	19.4	19.0	26.1	1.47
Date × genotype	126	10.4	6.7	6.1	14.2	9.6	9.8	13.3	8.0	14.2	1.32
	3,821	–	–	–	–	–	–	–	–	–	–
GCA	7	38.4	7.9	51.7	72.2	18.3	41.6	102.6	58.7	72.2	2.64
SCA	28	29.3	27.9	16.6	29.4	18.7	10.3	10.4	14.6	29.4	1.70
Reciprocal	28	12.9	15.7	21.3	11.6	25.7	10.8	7.7	13.6	11.1	1.70
	3,821	–	–	–	–	–	–	–	–	–	–
Correlation P.O.	28	0.350	0.041	0.031	0.716	0.344	0.481	0.497	0.040	0.411	
h^2 (narrow sense)	–	0.310*	0.014 NS	0.016 NS	0.618**	0.297 NS	0.300*	0.320*	0.050 NS	0.300*	

* Significant at 5% level; ** Significant at 1% level

crosses. These differences are due to maternal effects. They are connected with the physiological status of the mother plant and with its cytoplasm.

The estimations of the heritabilities (narrow sense) are given in Table 1. Only when scutella are grown on medium P, are heritabilities significant for all characters analysed. This fact indicates that some media are certainly better as breeding material for in vitro characters.

Because the regeneration rate was not very high, regenerated plantlets were not obtained for all genotypes. It was therefore not possible to investigate so precisely the following steps of the regnerating process: the developmental process of the plantlets in tube, genotypic aptitude for rooting in agar or in soil. However, the heterosis estimated by the ratio of the hybrid value with the mid-parent appears to be low for the characters studied previously but seems to be higher for the growth characters of plantlets which have just been regenerated.

Experiment 2

The capacities to regenerate plants from three populations of calluses issued from three different populations of embryos were compared. At the fifth passage the pieces of callus were transferred onto the regenerating media and the development of young plantlets was observed. Because the heritability measured through the diallel trial was the highest using medium P, only this medium was used for this experiment. In Table 2 values of the Fisher test are given for the same characters as those studied in the diallel trial.

Table 2. Comparison of the three populations for the characteristics of in vitro culture

Characters	Value fo Fisher test between populations	Mean arrangement (5% confidence limits)
Shoot forming capacity	15.3**	T < BC < S
Root forming capacity	0.742 NS	T BC S
Callus growth	0.978 NS	T BC S

** Result significant at 1% level

It appears that the ability to produce shoots or shoot-like organs through breeding was improved while the root or root-like organ formation and callus growth were not modified by selection. In Table 3 percentage of scutella which have given at least one normal living plant (planted in soil) for each population are reported. There are significant differences between the populations for these characters. The regenerating rate is rather low in each case because it was evaluated after 5 months of culture.

Also the plants regenerated from the population S were less depressed than those regenerated from the other populations meaning the production of short stalked plants with terminal female inflorescences.

Discussion

In the diallel trial it was shown that the SCA is significant. Since it measures a part of dominance and epistasis, the information on the parents only cannot be used to predict hybrid value for in vitro aptitude. The in vitro characters are, however, heritable, but different values of heritability on different medium are found and the estimations made are lower than those found in other works examining the same kinds of characters (Frankenberger et al. 1981). While Frankenberger et al. did not find any reciprocal effect in tomato such effects were observed in maize in agreement with Keyes et al. (1980) in red clover and with Foroughi-Wehr et al. (1982) in *Hordeum vulgare*. Hence it is possible that genetic cytoplasmic factors are involved in the genetic determination of the in vitro characters.

The results of the breeding experiment indicate that it is possible to breed for in vitro aptitude with maize and particularly for the percentage of regenerating callus after a long time of in vitro culture. By breeding for the complete process of in vitro aptitude the vegetal material has already been improved.

Plant regeneration from tissue culture of *Zea mays* was first reported by Green and Philips in 1975 but the regeneration of whole plants is sporadic and limited to a few genotypes. The percentage of regenerating tissue decreases dramatically with duration of callus growth. All these facts are the main obstacles for the obtain-

Table 3. Comparison of the three populations for the percentage of regenerating scutellum

Population	No. of observed scutella	No. of scutella given at least one normal plant	Observed percentage and confident limits (5%)
T	132	7	5.3 (2.2 – 10)
BC	737	70	9.5 (6.8 – 11.8)
S	752	108	14.4 (12 – 16.8)

ment of an in vitro selection method at the cellular or callus level. So before trying to apply such techniques of selection for breeding purposes one must be able to regenerate a large number of plants without problem. Considerable work remains before these procedures can be considered as routine.

References

Beckert M, Pollacsek M (1979) Expression de la variabilité génétique du maïs (*Zea mays* L.) en différentes conditions de culture de tissus. Ann Amelior Plant 29:563–581

Beckert M (1982) Importance of the scutellum in obtaining in vitro regeneration of maize. Agronomie 2:611–616

Bingham ET, Hurley LV, Kaatz DM, Saunders JW (1975) Breeding alfalfa which regenerates from callus tissue in culture. Crop Sci 15:719–721

Fouroughi-Wehr B, Friedt W, Wenzel G (1982) On the genetic improvement of androgenetic haploid formation in *Hordeum vulgare* L. Theor Appl Genet 62:233–239

Frankenberger EA, Hasegawa PM, Tigchelaar EC (1981) Diallel analysis of shoot forming capacity among selected tomato genotypes. Z Pflanzenphysiol 102:233–242

Green CE, Phillips RL (1975) Plant regeneration from tissue culture of maize. Crop Sci 15:417–421

Griffing B (1956) Concept of general and specific combining ability in relation to diallel crossing systems. Aust J Biol Sci 9:463–493

Keyes GJ, Collins GB, Taylor NL (1980) Genetic variation in tissue culture of red clover. Theor Appl Genet 58:265–271