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Plant regeneration from mature embryos of maize *(Zea mays* L.)

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ABSTRACT

In order to evaluate the induction of callus capable of plant regeneration, scutellum, plumule, radicle, and embryonary nodal tissues excised from mature embryos of a commercial F1 hybrid of maize were cultured *in vitro*.
Six to 10 days after culture initiation, plumule tissues turned brown and lost viability. The same response was observed 15-20 days after sowing scutellar slices as explants. Only central node and radicles were adequate tissues for callus induction under the used conditions
After 90 days in culture, viables calli were transferred to regeneration medium
Plants were regenerated in 42 % of the central-node-induced calli (mean of two experiments). Radicle tissues only regenerated roots
It is herein reported a simple and efficient protocol to obtain plant regeneration from callus induced from central node of maize mature embryos.

Keywords: *Zea mays*, mature embryos, *in vitro*, plant regeneration

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RESUMEN

Escutelo, plúmula, radícula y tejido nodal de embriones maduros de un híbrido comercial F1 de maíz fueron cultivados *in vitro* para evaluar la inducción de callos capaces de regenerar plantas.
Entre 6 y 10 días después de iniciados los cultivos, los tejidos de la plúmula se amarronaron y murieron. Lo mismo se observó entre 15 y 20 días luego de la siembra para las porciones de escutelo. En las condiciones ensayadas, sólo la región del nudo embrionario y de las radículas fueron explantos adecuados para inducir callos.
Luego de 90 días en cultivo, al transferir los callos al medio de regeneración, se obtuvieron plantas regeneradas del 42 % (promedio de dos ensayos) de los callos viables inducidos a partir de la zona nodal. Los callos formados a partir de radículas diferenciaron sólo raíces en todos los casos
Se presenta en este trabajo un protocolo simple y eficiente para obtener regeneración de plantas de maíz a partir de callos inducidos del nudo central de granos maduros

Palabras Clave: *Zea mays*, embriones maduros, *in vitro*, regeneración

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Plant regeneration from *in vitro* cultured tissues of maize has been succesfully performed using many different explants such as mesocotyl (Torné *et al.*, 1980), immature tassels (Rhodes *et al.*, 1986), glumes (Suprasanna *et al.*, 1986), leaf segments (Ray and Ghosh, 1990), and mature (Green *et al.*, 1974; Sheridan, W., 1975; Beckert and Pollacsek, 1979) or immature embryos (Green and Phillips, 1975).

Immature embryos are the most frequently used explants. They are often referred to as the best source for initiating cell suspensions or protoplasts cultures derived from calli grown on solid media (Green, C., 1982; Vasil and Vasil, 1991). However, they can only be obtained during a short period after flowering. This fact imposes a delay of 60 or more days to initiate new experiments. This condition could be obviated by using mature embryos, if the cultures are observed to possess morphogenetic potential.

The developmental status of the explants is a decisive factor involved in the morphogenetic response of calli cultures (Lu *et al.*, 1983; Vasil and Vasil, 1991). The use of germinating mature embryos as alternative tissue source would provide more reproducible working conditions: environmental effects can be easily controlled during germination and, accordingly, the physiological status is not expected to differ significantly among separated experiences.

In the present study, scutellar, plumule, radicle and nodal tissues were dissected from germinating kernels and were assayed separately. By this methodology, the interactions among different embryonary tissues and/or possitional effects during *in vitro* culture could be ignored and, furthermore, the better responding, specific tissues were identified.

The objective of this work was to investigate induction and regeneration capacity of calli derived from different tissues of germinating mature embryos (16-20 hs from imbibition).

The genotype considered in this experience was a commercial sweet F1 hybrid type of corn: Epi d'or, from Vilmorin Semmences, France.

Thirty kernels were surface-sterilized with 0,1% mercuric chloride for 10 min, then washed 3 times with sterile distilled water. The operation was repeated with a 2% active-chlorine solution plus 2 drops of detergent for 15 minutes. Finally the material was washed 3 times. Seeds were put into 125 ml erlenmeyer flasks containing 30 ml of sterile water, and left overnight in a giratory shaker (100 rpm) at 24 ± 2°C in the dark. The surface disinfection with 2% chlorine was repeated on the following day. Kernels were kept in sterile Petri dishes while embryonary axis were dissected under 50x magnification. The coleoptile and coleorhiza were removed so that exposed plumule, radicle and central node were excised and placed separately on culture media. Thin slices of scutellar tissues were also cut and placed in culture.

One standard culture medium was employed for callus initiation and mainteinance: MS (Murashige and Skoog, 1962), supplemented with 0,5 $g.l^{-1}$ of caseine hydrolisate, 100 $mg.l^{-1}$ myo-inositol, 100 $mg.l^{-1}$ thiamine-HCl, 1,38 $g.l^{-1}$ of L-proline and sucrose 30 $g.l^{-1}$. The growth-regulator used was 2,4-D (2,4-Dichlorophenoxyacetic acid) at 1 $mg.l^{-1}$. The pH was adjusted to 5.8 before the addition of 7 $g.l^{-1}$ of agar (Sigma Chem. Co.) and 20 ml of media were dispensed into 100 ml capacity flasks before autoclaving at 0.101 MPa for 15 min.

Cultures were located in the dark at 24 ± 2°C for callus induction and were transferred to fresh medium every 10-12 days during 3 months (total period of growth). In order to obtain plant regeneration, the cultures were then transferred to the same medium lacking 2,4-D, and kept under a 16 hs/day photoperiod (57 $\mu E.h^{-1}.m^{-2}$) and at 24 ± 2°C of temperature. Twenty or more days later, regenerated plants were transferred to pots filled with sterilized vermiculite and soil (1:1) and placed in humid chambers for 7-10 days until they were transplanted to soil pots in the greenhouse.

The experiment was repeated twice and very similar results were obtained in both trials. Results are summarized in Table 1

Callus initiation was evident after 4-5 days of culture in 2 of the 4 explants: radicles and central node. Plumules and scutellar tissues did not show any progress and shortly afterwards they turned brown and died.

After 15-20 days in culture, few scutellar explants which had initiated callus formation ceased growing, turned brown and died. Few of these explants were maintained for some weeks by means of frequent passages to fresh medium, but, anyway, they were further lost. It should be proved in the future whether these losses could be avoided by the addition of cytokinins or of other auxins to the culture medium. However, the low growth rate of these explants when comparing them to other assayed tissues indicates a preliminar resistance to this idea.

Radicle and central node were the only competent tissues, taken from mature kernels, so as to establish viable cultures of maize callus.

During the regeneration phase it was observed that radicle-induced callus differentiated only roots when 2,4-D was removed: no shoot formation occurred in these cultures. In the case of central node-induced-callus, plant regeneration was observed at a relatively

Table 1. Frequencies of callus induction and plant regeneration from different tissues of maize mature embryos in 2 experiments (in rows).

Tissue	Explants	Callus	Freq.%	Regenerated	Freq.%
Plumule	20	none	0	-	0
	23	none	0	-	0
Scutellum	24	none	0	-	0
	28	none	0	-	0
Radicle	18	11	60	8 (roots)	44
	24	15	62	6 (roots)	40
C. Node	26	22	85	13 (plants)	50
	23	17	73	8 (plants)	35

high frequency: 13/ 26 in experiment 1 and 8/ 23 in experiment 2, representing a 42 % mean frequency of plant regeneration. When the net ratio. regenerated plants/ number of viable cultures at the end of the growth period is considered, the mean frequency reaches 49% .

Many plants were lost during the hardening period in humid chambers (7/ 21 = 33% in experiment 1 and 5/ 11 = 45% in experiment 2), showing that this part of the experiment is still an important limiting factor of the process as a whole.

In previous reports of Green and Phillips (1974), Sheridan (1975) and Beckert and Pollasek (1979), mature embryos have been used for *in vitro* culture of maize. It was a common observation in these studies that cultures mantained on 2,4-D did not require cytokinins for growth, and that callusing initiated in the apical meristematic region of the embryo. Nodal tissues, assayed in this report, correspond to this same region and results obtained show that the central node from germinating mature maize kernels can be considered a suitable tissue source, in order to establish callus capables of plant regeneration.

Santos *et al.* (1984) observed a low capacity of totipotency for nodal-meristem induced callus when using germinating mature embryos of maize Their final consideration was that "mature kernels germination is no ideal for plant regeneration *in vitro* in spite of positive results in some cases". It is to note that, beyond the procedures described by these authors, vegetative development of the seedling is not inhibited but, on the contrary, it is simultaneous with callus proliferation. This difficulty to handle the cultures does not take place when the kernels are dissected. Besides, the interactions among different tissues of the germinating embryos, which may affect the response of competent tissues, can be ignored by using the present technique. This indicates that the reported procedure can be considered as an improved method to regenerate plants from maize mature embryos.

A dissection technique was also performed by Wang (1987). He induced callus from plumule tissues of maize mature embryos and reported that plant regeneration occurred at low frequencies (4-5%).

In the present work plumule explants gave a null response in a regeneration assay. However, the frequency of plant regeneration was high (42%) for nodal-induced calli. It is possible that the aleatory presence of nodal tissues in Wang's plumule explants were responsible for his reported low frequency of plant regeneration. Otherwise, these differences might be explained in terms of genotypic effects for different *in vitro* aptitudes of maize strains.

In order to evaluate this possibility, research work with other maize genotypes is under progress

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REFERENCES

1 Beckert M. and M. Pollacsek, 1979. Expression de la variabilité génétique du Mais (*Zea mays* L) en differentes conditions de culture de tissus Ann. Amélior. Plantes 29: 563-581

2 Green C E. and R.L Phillips, 1975 Plant regeneration from tissue cultures of maize Crop Sci. 15· 417-421

3. Green C.E., R.L. Phillips and R.A. Kleese, 1974. Tissue cultures of maize (*Zea mays* L.): initiation, manteinance and organic growth factors. Crop Sci. 14· 54-58.

4. Green C.E., 1982. Somatic embryogenesis and plant regeneration from the friable callus of *Zea mays*. In: Plant tissue culture 1982 (Proc. V Int. Congr. Plant Tissue and Cell Culture) Fuyiwara, A. ed. Jap. Assoc. Plant Tissue Culture, Tokyo, Japan. pp 107-108.

5. Lu C., V. Vasil and I.K. Vasil, 1983. Improved efficiency of somatic embryogenesis and plant regeneration in tissue cultures of maize (*Zea mays* L.). Theor. Appl. Genet. 66. 285-289.

6. Murashige T.T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Phys Plant. 15: 473-497.

7. Ray D. S and P.D. Gosh, 1990. Somatic embryogenesis and plant regeneration from cultured leaf explants of *Zea mays*. Ann.Bot.66:497-500

8 Rhodes C.A., C.E. Green and R.L. Phillips, 1986. Factors affecting tissue culture initiation from maize tassels Plant Science 46: 225-232.

9. Santos M.A., J.M. Torné and J.L. Blanco, 1984 Methods of obtaining maize totipotent tissues I.Seedling segments culture. Plant Sci. Lett. 33. 309-315.

10. Sheridan W.F., 1975. Tissue culture of Maize. I:Callus induction and growth. Physiol. Plant. 33· 151-156

11. Suprasanna P., K.V.Rao and G.M.Reddy, 1986. Plantlet regeneration from glume calli of maize (*Zea mays* L.).Theor.Appl.Genet. 72: 120-122.

12. Torné J.M., M.A. Santos, A. Pons and M. Blanco, 1980. Regeneration of plants from mesocotyl tissue cultures of immature embryos of *Zea mays* L. Plant Sci. L. 17· 339-344.

13. Vasil I.K. and V. Vasil, 1991. Embryogenic callus, cell suspension and protoplast cultures of cereals In: Plant Tissue Culture Manual (suppl. 2) K Lindsey (Ed).Kluwer Pub., Dordrecht

14. Wang A.S., 1987. Callus induction and plant regeneration from maize mature embryos. Plant Cell Rep. 6· 360- 362.