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Plantlet regeneration from glume calli of maize (*Zea mays* L.)

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Summary. Totipotent callus cultures were established from anther-free glumes of 'Sweet corn', 'Seed corn,' 'DHM 103' and 'DHM 101' on MS medium supplemented with 1–2 mg/l 2,4-D. The callusing response of the glumes was tested on six different media. Glumes at the uninucleate stage of pollen development callused with a high frequency compared to other stages. Organogenesis was observed in 40% of the cultures on media devoid of hormones. A total of 76 plantlets were regenerated on medium with 0.5–1.0 mg/l of both IAA and kinetin. Cytological observations in root tips indicated a diploid chromosome number (2n = 20).

Key words: Maize glumes – Callus initiation – Plantlet regeneration

Introduction

In addition to genetic manipulation in plants, plant cell and tissue cultures are increasingly being used as important tools in the study of biochemical and molecular processes. In maize, early studies dealt with the culture of sugary (La Rue 1949; Straus and La Rue 1954) and non-sugary endosperm (Coe and Reddy 1961). This was followed by reports on callus initiation and maintenance from diploid sources (Mascarenhas et al. 1965), and successful planlet regeneration from scutellar cultures (Green and Phillips 1975) and from mesocotyl sections of germinated immature embryos (Torne et al. 1981).

Callus initiation and plantlet regeneration from different explants with variable gene expression, by utilizing specific genetic markers, may help in understanding the biosynthesis of anthocyanin and its regulation at the cellular level. The present study mainly deals with the totipotency of glumes for callus initiation and plantlet regeneration.

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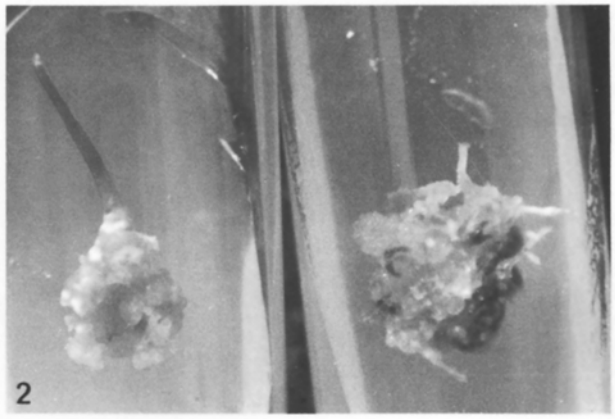
Materials and methods

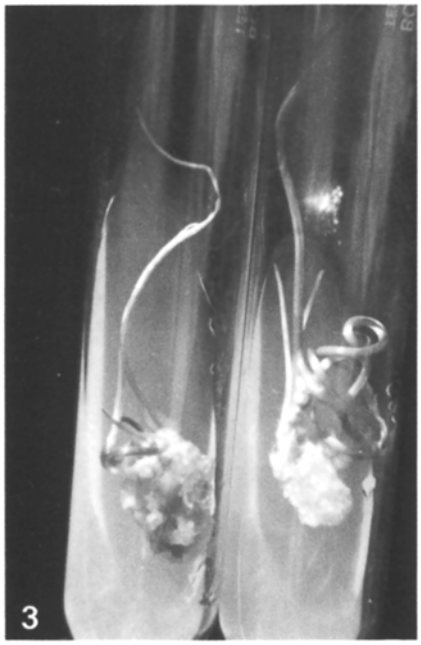
Four locally grown cultivars of maize namely 'Sweet corn', 'Seed corn', 'Deccan Hybrid Macca 103' and 'Deccan Hybrid Macca 101' were used in the present study. Fresh immature tassels, about 7–15 cm long, enclosed in leaves, were collected and sterilized with 0.1% mercuric chloride for 3 min followed by thorough washing in sterile distilled water. Anthers were removed and the glumes were inoculated onto 6 media: Blaydes (1966); B5 (Gamborg 1968); Linsmaier and Skoog (1965); Murashige and Skoog (1962); Nitsch and Nitsch (1969) and White (1954), supplemented with 2% sucrose and 0.5–4.0 mg/l 2,4-dichlorophenoxy acetic acid (2,4-D). The stage of pollen development in the anther, detected by staining with acetocarmine, was used as an index for the age of the glumes. About 5–7 glumes were inoculated per test tube. The total number of explants used varied from 500 to 1,000 for each genotype.

Two weeks after callus initiation, callus without any residual explant was subcultured on MS basal medium with 2,4-D. MS basal medium devoid of hormones and medium supplemented with indole 3-acetic acid (IAA) and kinetin 0.5–2.0 mg/l were used for plantlet regeneration. A total of 150 cultures were incubated under continuous flourescent light (800–1000 lux) at 26±1 °C.

Results and discussion

About two weeks after inoculation, callus initiation was observed at the base of the glume (Fig. 1). Callus formation in some inocula was poor and exhibited rhizogenesis. Glumes collected at the uninucleate stage





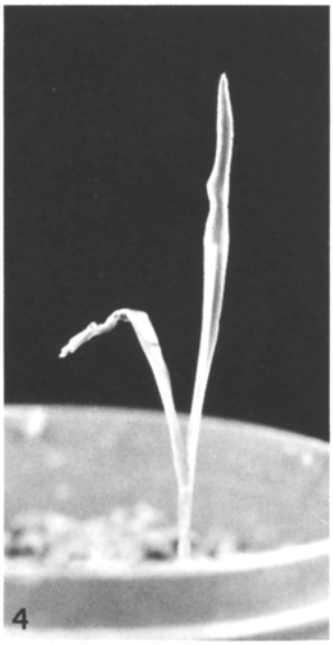


Fig. 1. Callus initiation from glumes on MS medium with 2.4-D (2 mg/l)

Fig. 2. Organogenesis in glume callus on MS basal medium devoid of hormones

Fig. 3. Ten-day-old plantlet growing on MS medium with IAA (1 mg/l) and kinetin (1 mg/l)

Fig. 4. Regenerated plantlet transplanted into vermiculite pot

of microsporogenesis exhibited a greater response in callus initiation compared to those collected at the binucleate stage (Table 1). The four maize cultivars differed in time taken for callus initiation and in efficiency for callusing (Table 1). At the uninucleate and binucleate stages of pollen development, callus induction was maximum in 'Sweet corn' and 'Seed corn' followed by 'DHM 103' and 'DHM 101'. Apparently callus induction can not be observed in 'DHM 101' at the binucleate stage.

Of the six media containing different concentrations of 2,4-D, MS medium supplemented with 2 mg/l 2,4-D was effective in callus induction (Table 1) compared to other media: LS (9%), B5 (11%), Blaydes (7.5%), Nitsch and Nitsch, and Whites (0%). Other concentrations of 2,4-D (0.5, 1.0 and 4.0 mg/l) had no significant effect on callus initiation.

Subcultures were usually carried out every 4 weeks on the same media. By two to three weeks after transfer to MS regeneration medium devoid of hormones, callus growth was rapid and greening was observed in the callus (Fig. 2) leading to the formation of small leaf-like structures and even shoots (Fig. 2). The differentiation into complete plantlets, however, was accomplished by transferring these cultures onto regeneration medium containing 1 mg/l IAA and 1 mg/l kinetin (Fig. 3). Cytological examination of the root tips of plantlets indicated 20 chromosomes in each.

Calli of 'Sweet corn' showed a high regenerating ability compared to other cultivars (Table 1). The regenerating ability continued after 205 days. A total of 76 plantlets were transferred onto vermiculite pots (Fig. 4) supplemented with sterilized soil and 25% strength MS liquid medium. Plantlets from the three cultivars grew to 20–30 cm after which leaves showed necrosis and did not survive. Further studies on the survival of regenerated plantlets are in progress.

Rhoades et al. (1982), in culturing tassel sections from the basal half of the inflorescence, found the highest response for callus initiation (82%), suggesting that the section size may

Table 1. Frequency of callus initiation and plant regeneration from glume cultures of *Zea mays* L.

Genotype	Stage[a] of pollen for glume cultured	Time (days)	Callus initiation (%)	Plantlet regeneration (%)
Sweet corn	Un	10–12	64.5	34.0
Sweet corn	Bn	18–20	27.8	16.5
Seed corn	Un	13–16	60.1	26.0
Seed corn	Bn	20–22	18.0	6.6
DHM 103	Un	18–20	30.2	22.2
DHM 103	Bn	18–25	12.4	4.3
DHM 101	Un	20–25	14.4	–
DHM 101	Bn	–	–	–

[a] Un = uninucleate; Bn = binucleate

play an important role in determining optimal cultural conditions. In the present study, callus formation was observed from glumes of 7–15 cm long tassels in which the pollen development stage was used as an index for the age of glumes. The observations suggest that, in addition to tassel size, the age of the glume also plays an important role in callus induction and plantlet regeneration.

Although many of the cereals have been cultured, plant regeneration represents the prime factor in the utilization of in vitro culture techniques for further studies. In maize, only scutellum derived cultures have been the source of totipotency, especially from A 188 inbred line and its hybrids (Lu et al. 1983).

For callus induction from glumes, 2,4-D has been the source of auxin and other hormones such as IAA, NAA and KN had no effect. Maize embryos apparently do not require cytokinins for callus initiation (Green and Phillips 1974) whereas endosperm require no auxins for callus initiation and growth (Sheridan 1975), which reflects the differential response of tissues cultured in vitro to exogenous hormones. In our study, although organogenesis was observed on basal medium without any hormone, plantlet regeneration could be achieved only with IAA and kinetin, which suggests that the combination or the interaction of auxins and cytokinins may be required for complete differentiation into plantlets.

The age of the glume played an important role in callus induction. Glumes taken at the uninucleate stage of the microspores callused better compared to the poor response found at the binucleate stage (Table 1). This shows that rapidly growing tissue is conducive for callus formation and subsequent plantlet regeneration.

It is clear, however, that not all lines of maize respond similarly and can be established in culture (Sheridan 1975). In the culture of endosperm tissue, only sugary types like 'Black Mexican Sweet' gave successful callus induction and growth (Straus and La Rue 1954). The four maize cultivars used in this study differed considerably in their response for callus initiation and plantlet regeneration. 'Sweet corn' showed the highest response (34.0) followed by 'Seed corn', 'DHM 103' and 'DHM 101', both in the uninucleate and binucleate stages. The differential response among the four maize cultivars may be due to genotypic differences.

Fresh developing glumes offer an efficient source of material for callus initiation and plantlet regeneration not only in maize but also in other crop plants which offer great potential in the study and maintenance of male steriles and aneuploids. Studies using convenient genetic markers which control pigmentation in glumes are in progress in order to understand the mechanism of gene regulation in anthocyanin biosynthesis.

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