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INDUCTION OF DIRECT SOMATIC EMBRYOGENESIS FROM IMMATURE ZYGOTIC EMBRYOS AND CALLOGENESIS FROM EPICOTYL EXPLANTS OF *MORINGA PTERYGOSPERMA* GAERTN.

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Direct somatic embryogenesis was obtained from immature zygotic embryos of *Moringa pterygosperma* Geertn. cultured in continuous light in media with GA_3, BAP and activated charcoal. Long term, fast-growing callus cultures were established from rapidly elongating epicotyls of *in vitro* plantlets of *Moringa* in media with 2,4-D, NAA and coconut milk.

Keywords : *Epicotyl callus; Moringa; Somatic embryogenesis.*

Introduction

Moringa pterygoserma Gaertn. is the source of drumstick and is also useful in reforestation and water treatment[1]. It is of medicinal importance as it possesses anti-tumour[2] and hypoglycaemic[3] activities. Isothiocyanate and rare thiocarbamate glycosides[4] with hypotensive effect have been isolated from *Moringa*. The tree is mainly propagated vegeratively as the seeds lose their viability soon. There is a need to apply tissue culture techniques for increasing the productivity of this tree and its genetic improvement. There are only two[5,6] reports on the *in vitro* propagation of *Moringa* and these pertain to the culture of nodal explants[5] and hypocotyl and cotyledonary explants[6]. The poor germination of mature seeds limits their use for obtaining *in vitro* plantlets as explant sources. In the present investigation immature zygotic embryos and seeds from fruits of *Moringa* were cultured to ascertain their potential for germination *in vitro* to yield contaminant-free experimental material and also for somatic embryogenesis which is an useful alternative method for *in vitro* propagation. The present study reports direct somatic exbryogenesis from immature zygotic embryos of *Moringa* and the establishment of fast-growing, long term callus cultures, from rapidly elongating epicotyl explants, which may serve as stable sources of medicinal compounds of *Moringa.*

Materials and Methods

Seeds (0.8 cm - 1.5 cm) in diameter were aseptically extracted from mature fresh green fruits of *Moringa* and were dewinged. After partial or total removal of seed coat the zygotic embryos were cultured on Murashige and Skoog[7] (MS) medium with or without hormones (Table 1) and with activated charcoal (0.25%). All cultures were incubated in continuous light under cool white fluorescent lamps at 25± 2°C.

Results and Discussion

Direct formation of tiny globular somatic embryos was seen after two weeks of culture, in medium B with GA_3 and BAP, at the radicular end of zygotic embryos from immature seeds (0.8 cm in diameter) in which the seed coat had been partially removed. After a further period of two weeks the somatic embryos enlarged and turned green and at five weeks of culture 4-6 somatic embryos at both globular and cotyledonary stages were visible (Figs.1 and 2). The frequency of somatic embryogenesis was about 20%. The induction of direct somatic embryogenesis without any callus phase is highly significant since these

propagules are genetically indentical to the explant. No somatic embryogenesis could be induced in zygotic embryos from seeds of 0.8 cm diameter in media without hormones and zygotic embryos from larger seeds (1.5 cm in diameter) in media with hormones (Table 2). These observations reflect the key role[8] played by hormones in somatic embryogenesis and corroborate earlier reports[9,10] that immature zygotic embryos have greater potential for somatic embryogenesis than mature zygotic embryos. Direct somatic embryogenesis from zygotic embryos has also been reported in other woody plants such as *Prunus avium*[10]. There are only a few reports of induction of somatic embryogenesis by GA_3[11,12] in angiosperms and the involvement of GA_3 along with cytokinins in the induction of somatic embryogenesis in *Moringa* observed here corroborates earlier observations[13,14] where a high level of these hormones was associated with the initial stages of fruit development and therefore embryogenesis. Activated charcoal has been associated with somatic embryogenesis in cassava[15]. 2,4-D which causes genetic changes[16] esd not required for somatic embryogenesis in *Moringa*.

Germination *in vitro* and plantlet formation was obtained from immature zygotic embryos, 0.8 cm in diamerer,

Table 1. Growth regulators (mg/1) and other supplements added to different media.

Components	A	B	C	D	E
BAP	–	1	–	3	2
2, 4-D	–	–	4	–	–
GA_3	–	1	–	–	–
NAA	–	–	1	–	0.2
Coconut milk (%)	–	–	15	–	–
Activated charcoal (%)	0.25	0.25	–	–	–

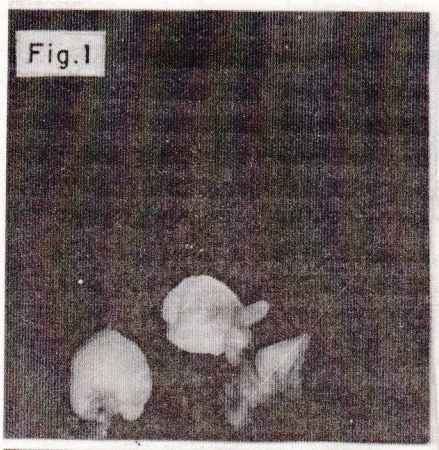
Table 2. Responses of zygotic embryos from seeds of different diameters.

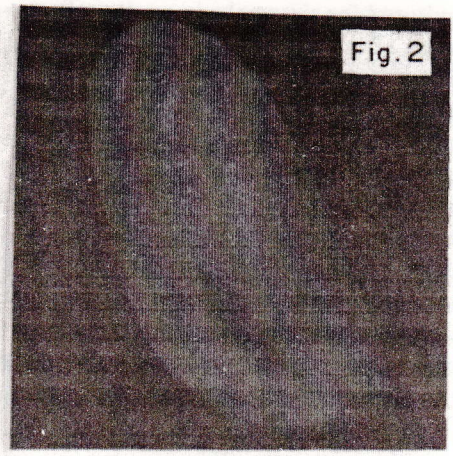
Diameter	Responses
0.8 cm	1. Somatic embryogenesis in medium B
	2. Formation of *in vitro* plantlet with vigorously elongating epicotyl in medium B
1.5 cm	Germination in media A and B but with poor root formation and shoot axis with stunted growth

Table 3. Growth of epicotyl callus in different media.

Medium	Growth of callus
C	++++
D	+
E	+

+ – – Relative measure of callus growth.





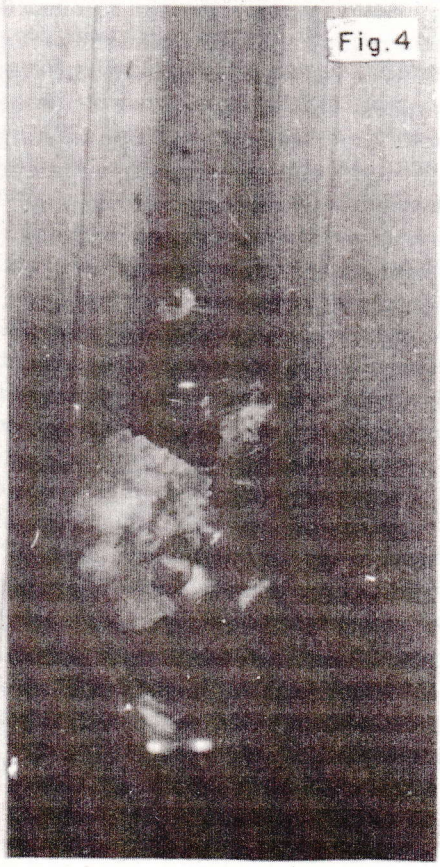


Fig.1. Somatic embryos (arrowed) at both globular and cotyledonary stages in medium B;

Fig.2. Somatic embryo at cotyledonary stage (12X);

Fig.3. *In vitro* plantlet from immature zygotic embryo cultured in medium B;

Fig.4. Callusing on epicotyl explants in medium C.

cultured for six weeks in medium B, one week after transfer to fresh medium of the same composition. The epicotyl elongated rapidly and attained a length of 10 cm (Fig. 3) in a span of about twelve days and there was induction and rapid elongation of roots. Though zygotic embryos from larger seeds (1.5 cm diameter) germinated in media A and B the root growth was stunted and the shoot axis attained only a length of 3.5 cm and their was no further elongation or growth even on transfer to fresh media.

Explants from different parts of the *in vitro* plantlet obtained in medium B from zygotic embryos were cultured for callus induction. Callus induction was obtained only form explants from the basal part of the rapidly elongating epicotyl cultured in medium C. The induction of callogenesis from epicotyl has not so far been reported in *Moringa*. The fast growing callus was soft, amorphous, watery and transluscent (Fig. 4) and could be maintained after several passages of subculture over an year in medium C. The growth of the callus was maximum in medium C and was less in media D and E (Table 3). The fast-growing callus can be used as a stable source of the medicinal compounds of *Moringa* and the biosynthetic potential[17] of the callus can be investigated by adding suitable precursors.

The results of the present study are therefore significant in that induction of direct somatic embryogenesis could be achieved.

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