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# *In vitro* PROLIFERATION OF NUCELLUS TISSUE IN *Mangifera indica* L. var. *Zardalu AND M. indcia* L. var *Sepia*

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Key words : *Mangifera Indica* var. *Zardalu* and *Sepia*, nucellus, somatic embryogenesis

Nucellus, anthers and floral buds of *Mangifera indica* L. var. *Zardalu* and *M. indica* L. var *Sepia* were cultured on Murashige and Skoog's medium supplemented with various growth hormones either individually or in different combinations. We report here formation of granular and subsequently callus like proliferation on nucellus explants in MS liquid medium supplemented with 2, 4-D, NAA, IAA, BAP and Kinetin after 3-4 days of culture in both varieties. The proliferation was vigorous and spread all over the surface of cultured nucellus tissue. The subculture of explants to the fresh medium stimulated formation of undifferentiated homogenous mass of cells as well as somatic embryo. The early development of cultured nucellar explants show similar morphogenetic stages during proliferation of nucellus tissue in *Zardalu* and *Sepia* varieties of mango.

## INTRODUCTION

*Mangifera Indica* L., commonly known as mango, is one of the most important fruit crops and occupy half of the total area under cultivation in our country. Mango is an arborescent evergreen tree living from 70 to 100 years. Indian cultivars are usually monoembryonic and are propagated by grafting on polyemryonic seedling rootstock. Under normal condition a grafted tree produces 300-500 fruits in 10th year, about 1000 fruits in 15th year and 2000 to 5000 fruits from 20$^{th}$ year onwards. In nature monoembryonic cultivars have originated from seedlings derived from uncontrolled pollination and the complex set of genes determine the good horticultural characterstics in mango. *Zardalu* cultivar which is endemic to Champaran region of North Bihar (India) is highly delicious mango variety famous for its aroma and flavour. Both *M.indica* var. *Zardalu* and var. *Sepia* are facing biotic and abiotic stress posing threat to survival. Keeping in view the horticultural problems associated with mango cultivation, such as long juvenile phase, yield, low frequency of somatic mutation as well as several difficulties in breeding programmes faced by horticultural scientists and biotechnologists, it is highly desirable to use *in vitro* tissue culture technique for induction of somatic embryogenesis and mass cloning of the two selected elite varieties of mango.

In the case of polyembryonic or monoembryonic varieties of mango nucellus tissue were used as explant for induction of somatic embryogenesis (Litz *et al.*, 1982, 1998; Litz 1984; De Wald, 1989; Patena *et al.*, 2002; Chaturvedi *et al*., 2004; Kidwai *et al.,* 2009; Bimal and Singh, 2017). However, direct somatic embryogenesis was reported from cotyledon of mango in mature zygotic embryo (Xiao *et al.* 2004). In the present paper we report induction of nucellar proliferation in the two cultivars of mango, *M. indica* L. var. *Zardalu* and *Sepia*.

## MATERIAL AND METHODS

Explants were collected from Motipur mango orchards (Figs. 1-3) and different types of explants, *viz*., floral buds and fruits of *Zardalu* and *Sepia* were collected. Young immature fruits ranging in size from 0.2 cm to 3 cm (Fig. 4) were collected for experiment. They were washed thoroughly with tap water, liquid detergent followed by washing with distilled water. Washed fruits were surface sterilized with 2% $HgCl_2$ for 10 minutes and rinsed with sterile double distilled water 3-4 times. The sterilized fruits were cut longitudinally into two halves and the intact fertilized ovule was removed and nucellar tissues were taken as explants and were treated in wash mixture for one hour and finally these explants were surface sterilized again using 2% $HgCl_2$ solution for 5 minutes and rinsed with sterile double distilled water 3 to 4 times. The explants were inoculated in MS medium supplemented with various growth adjuvants.

Explants were first inoculated in double distilled water with 2, 4-D + BAP for 10 to 15 days. Later, the explants were transferred to MS (1962) basal nutrient medium either alone or the basal medium supplemented with growth hormones such as auxins or cytokinins individually or in combinations. The cultures were incubated in culture room at 25°C ± 5°C in dark.

## RESULTS AND DISCUSSION

The nucellus explants (Fig.5) of monoembryonic *Mangiera indica* L. var. *Zardalu* and *Sepia* were cultured under different nutritional conditions. The nucellus explants were cultured in medium $M_1$ containing plain sterile water supplemented with 2,4-D and BAP for 7-10 days in dark (Fig. 6). While in other set of experiment the nucellus explants were cultured in medium $M_2$ containing MS supplemented with 2,4-D and BAP for 7-10 days in dark. In both the cases, the nucellus explants showed induction of proliferation. The nucellus explants cultured in $M_1$ medium retained its white creamy colour for 7-8 days (Fig. 6), while the nucellus explants cultured in $M_2$ medium turned dark in 3-4 days (Fig.7). Interestingly, in 2-3

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days of culture nucellus explants cultured in the presence of 2, 4-D and BAP produced globular outgrowths over the whole surface in both the varieties of mango used for experiment (Fig. 8). For further growth and differentiation the explants were transferred to liquid MS medium supplemented with 2, 4-D ($1mgl^{-1}$) and KN ($1mgl^{-1}$) (Fig. 9-12).

In 4-6 week of culture in dark, whole surface was covered with layer of proliferated cells which were dark in colour. These on transfer to fresh medium showed growth and differentiation. Several groups of organised formations were also visible. In another 4-8 week of culture the proliferated surface started producing Pro-embryonic callus (PEC) like structure which further produced white outgrowths which looked like somatic embryo.

It is interesting to note that nucellar proliferations started in 3-4 days and became prominent in 10-15 days in 95 ±2% explants of both *Zardalu* and *Sepia* varieties of mango. After 4 to 6 weeks, differentiation of dark callus layer was also observed. Ara *et al.* (2000) also observed callus growth after 3-4 week of culture in 51.62 ± 13.67% excised nucellus explants. When the liquid nutrient medium turned dark due to phenolic secretion from cultured tissues, the explants were transferred to fresh medium. At least twenty replicates were maintained for each treatment and each treatment was repeated three times. In the present experimental systems differentiation of embryogenic callus or cells was observed which was also observed by Ara *et al.* (2000) and Kidwai *et al.* (2008). Xiao *et al.* (2002) found pre-conditioning of explants for 7-10 days necessary for induction of differentiation and morphogenesis in mango as also observed in *Zardalu* and *Sepia* varieties of mango. Fruit size ranging from 1.5 cm to 5.0 cm was found to be important for somatic embryogenesis in *Zardalu* and *Sepia* cultivars of mangoas observed by other workers in different mango varieties (Jana *et al.* 1994; Thomas 1999; Ara *et al.* 2000; Kidwai *et al.* 2009). The early morphogenetic events are highly *comparable* in *M.indica* var. *Zardalu* and *M. indica* var. *Sepia*. Further work is in progress for high frequency plant regeneration in *Zardalu* and *Sepia* mango varieties. The present study offers novel experimental system of endangered and endemic local mango germplasms of Bihar state (India) for conservation and improvement.

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**PLATE-1**

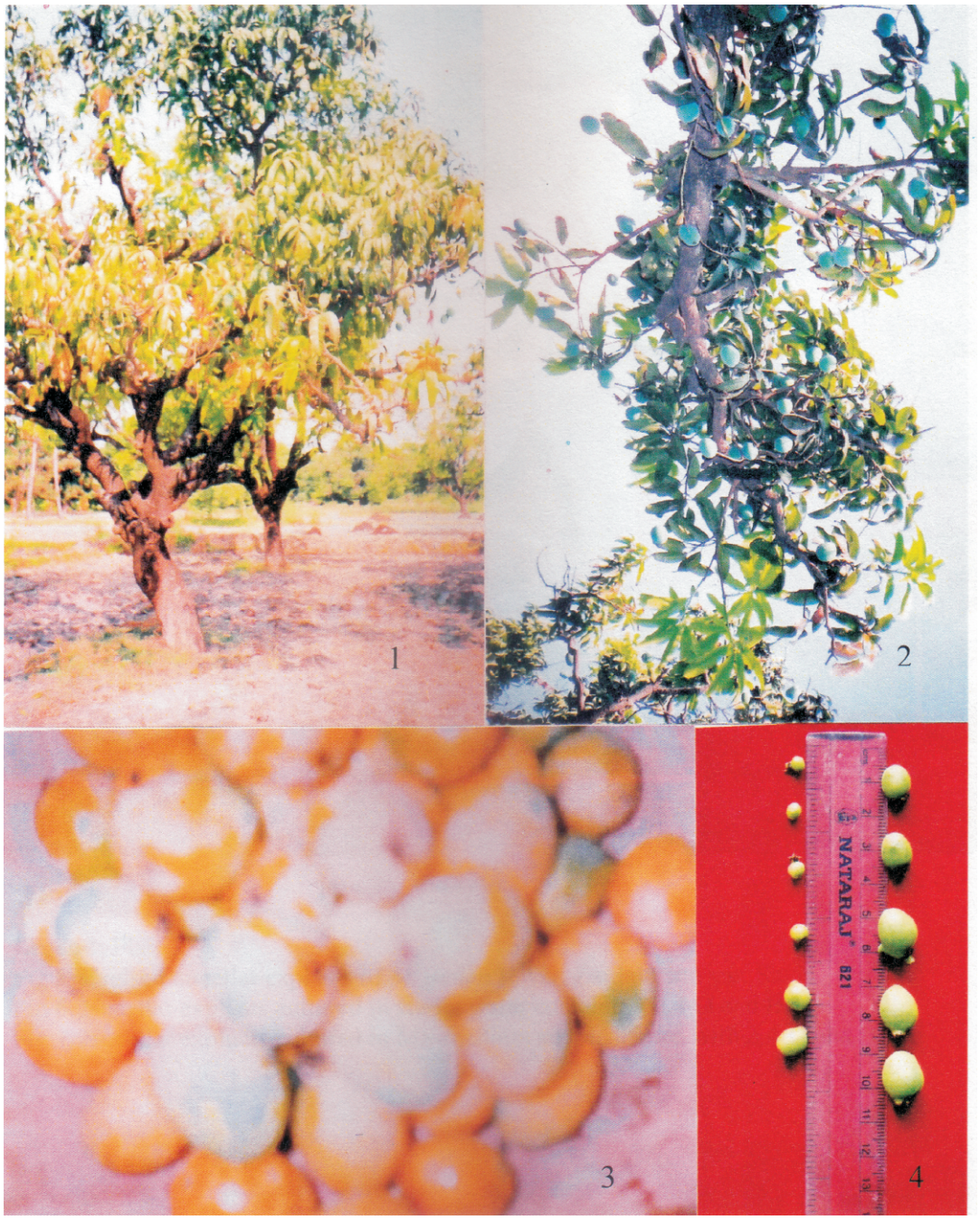


Fig 1. Mango orchard at Motipur (Muzaffarpur).

Fig 2. A fruit bearing branch of *M. indica* L.var *Zardalu*.

Fig 3. Ripe fruits of Mango var. *Zardalu*.

Fig 4. Young mango fruits of different size collected from field for experiment.

**PLATE-2**


Fig. 5. Excised nucellar explants of *M. indica* L. var. *Zardalu* and var. *Sepia*.

Fig. 6. Nucellar explants cultured in sterile water containing 2, 4-D and BAP.

Fig. 7. Nucellar explants cultured in MS- medium supplemented with 2,4-D and BAP.

**PLATE-3**


Fig. 8. The photograph showing proliferations on nucellar explants *M. indica* var. *Zardalu* after 8-10 days of culture on MS+2, 4-D 5mgl$^{-1}$ + BAP 0.25gml$^{-1}$.

Figs. 9-10. The photographs of histological preparations of proliferated surface showing development stage of somatic embryo.

Figs. 11-12. The photographs showing differentiation of shoots in M. indica var. *Zardalu*.