

IN VITRO PROPAGATION OF PRECOCIOUSLY GERMINATED SEEDLINGS OF *CARICA PAPAYA* L. VARIETY MADHUBINDU

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Abstract :- The precocious germination of seeds was observed in fruits of *Carica papaya* L. variety Madhubindu while they were still attached to the parent plant (vivipary), which is a native diversity observed in the local gene pool. In vitro culture study was done to maintain and propagate this variety because such native diversity by the large, face maintenance and conservation problems.

Key words : Pollinia, Orchid, diversity, SEM.

INTRODUCTION

Carica papaya L. variety Madhubindu bear fruits of medium size with good flavour and taste. In the present study the precocious germination of seeds was observed in fruits while they were still attached to the parent plants (vivipary), which is a native diversity observed in the local gene pool. The natural occurrence of precocious germination has been reported in papaya (Balakrishnan et. al., 1986). Native diversity by and large, face maintenance and conservation problems. It is thus essential to collect and evaluate the local gene pools. Propagation, conservation and maintenance of the diverse germplasm possessing specific traits, rootstock or good plant type is also required. *Carica papaya* L. variety Madhubindu showed natural occurrence of precocious germination of seeds in fruits, a diverse germplasm worth propagating.

In the present study all fruits on the same plant did not show vivipary (Fig. 1A). All seeds in a fruit did not show precocious germination (Fig. 1B). The seeds germinating precociously were in different stages of development (Fig. 1C). It has been reported that precocious papaya cultivars grown as annual

produce quick returns in field plantings (Ram, 1983). Tissue culture techniques would help in the rapid multiplication of this diversity. The present study was to grow the precociously germinated seedlings (i) directly on different substrates for hardening and then transferring them to field and (ii) on different media i.e. *in vitro* regeneration and growth. The *in vitro* culture studies also included generation of multiple shoots from the node and shoot apex explants from precociously germinated seedlings as a means of mass propagation (Fig. 1D).

MATERIAL AND METHODS :

In *Carica papaya* L. variety Madhubindu, total seeds and seeds germinating precociously were counted in ten fruits two each from five different plants. The precociously germinated seedlings were washed several times in distilled water treated with bavistine (0.5%) and then directly transferred for hardening to different substrate like soil, vermiculite, coco peat, soil; vermiculite (1 :1), Soil ; coco peat (1 : 1), soil : cocoa peat (1:1), vermiculite : coco peat (1:1), soil : vrmiculite : coco pear (1:1:1) in the hardening chamber having 26 1°C temperature and 90% relative humidity for 30 days. The temperature was gradually increased to room

temperature and relative humidity was reduced to 70%. The plantlets were then transferred to the green house after 30 days. The relative humidity was gradually brought down and maintained at 50% by using the overhead mist spray. Plants were kept in the green house for 30 days. Then the plants were transferred to the field.

For in vitro regeneration and growth, the precociously germinated seedlings were inoculated on MS (Murashige and Skoog, 1962), B5 (Gamborg's 1968), MS + B5 (Macro and micro salts of MS and vitamins of B5), W (Whites, 1963), LS (Linsmaier and Skoog, 1965) and WPM (Lloyd and McCown,) media. The in vitro grown precociously germinating seedlings

were the source of explants for further studies. Node and shoot apex explants from precociously Germinated seedlings were regenerated on different media (MS, B5, WPM, W, LS and MS + B5) fortified with auxins and cytokinins singly or in combination, supplemented with or without coconut milk. Cut ends of the regenerated multiple shoots were excised and dipped for 30 second in IBA (2500 mg/l), then inoculated on plain MS medium for rooting. The resultant, plantlets grew well in small pot containing soil, vermiculite and cow dung (1:1:1) mixture (Saha et. al., 2003). After hardening plantlets were transferred to field. The tissue-cultured plants grown in field were observed for seeds germinating precociously in fruits.

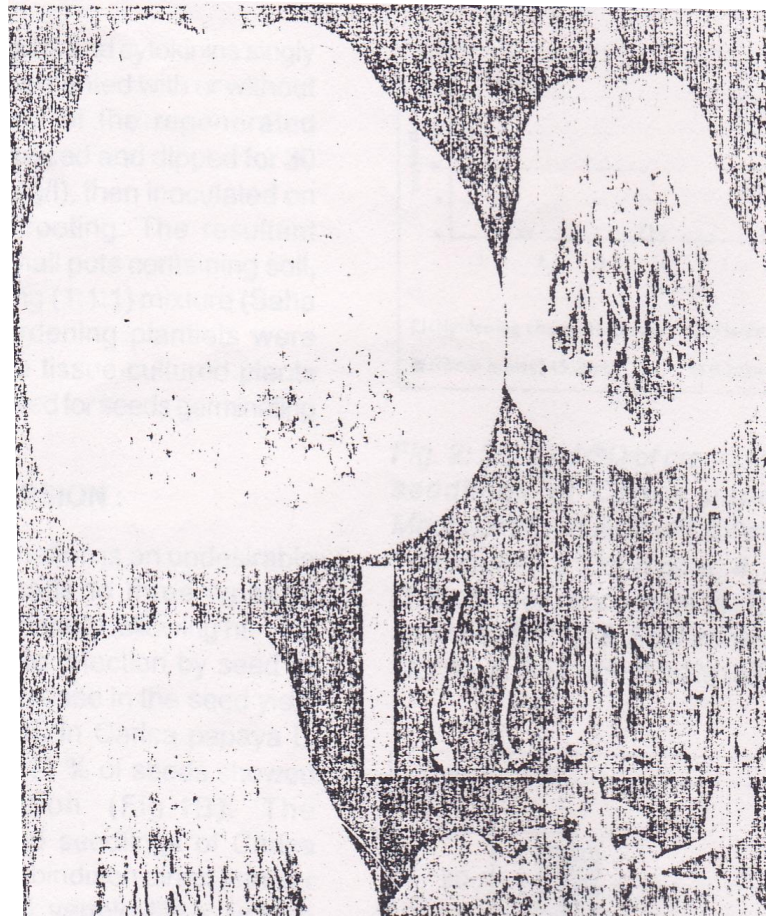


Fig. 1 : A. Fruits from the same plant, one without precocious germination of seeds and another with precocious germination of seeds. B. Carica papaya L. variety Madhubindu showing precocious germination of seeds in fruits . C Seeds in a fruit germinating precociously are in different stages of development. D. Multiple shoots from the node explants from precociously germinated seedlings.

RESULTS AND DISCUSSION :

Precocious germination is an undesirable phenomenon and it leads to three types of damage (i) loss of seed viability following harvest (ii) reduced resistance to infection by seed or grain mould and (iii) decrease in the seed yield (Ren and Bewley, 1998). In *Carica papaya* L. variety Madhubindu 78.11% of seeds showed precocious germination (Fig. 1B). The precociously germinated seedlings of *Carica papaya* L. variety Madhubindu on direct transfer to the combination of soil : vermiculite : cowdung (1:1:1) could result in 14% survival in 60 days (Fig. 2)

The precociously germinated seedlings of *Carica papaya* L. variety Madhubindu when inoculated on different media MS, B5, LS, W, WPM and MS + B5 showed 33.6, 15.3, 6.6, 14, 3.3 and 9.6 % survival respectively (Fig. 3)

Generation of multiple shoots from the node and shoot apex explants from precociously germinated seedlings served as a means for mass propagation. Node and shoot apex explants inoculated on MS + B5 and LS media fortified with auxins and cytokinins singly or in combination could not be established and on B5, W and WOM media fortified with auxins and cytokinins singly or in combination it showed either the development of excessive amount of callus or hypertrophy at the base.

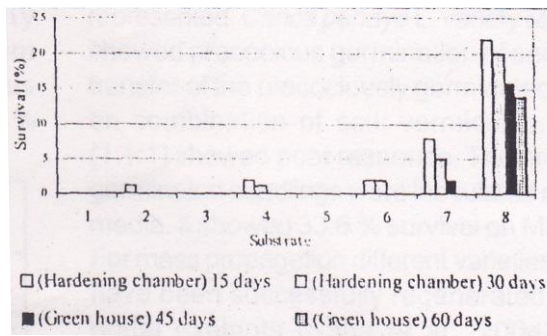


Fig. 2 : Survival (%) of precociously germinated seedlings of *Carica papaya* L. variety Madhubindu on different substrates - 1. Soil, 2. Vermiculite, 3. Coco peat, 4. Soil : vermiculite (1:1), 5, Soil : coco peat (1:1), 6. Vermiculite :

coco peat (1:1), 7. Soil : vermiculite : coco peat (1:1:1), 8. Soil : vermiculite : cowdung (1:1:1).

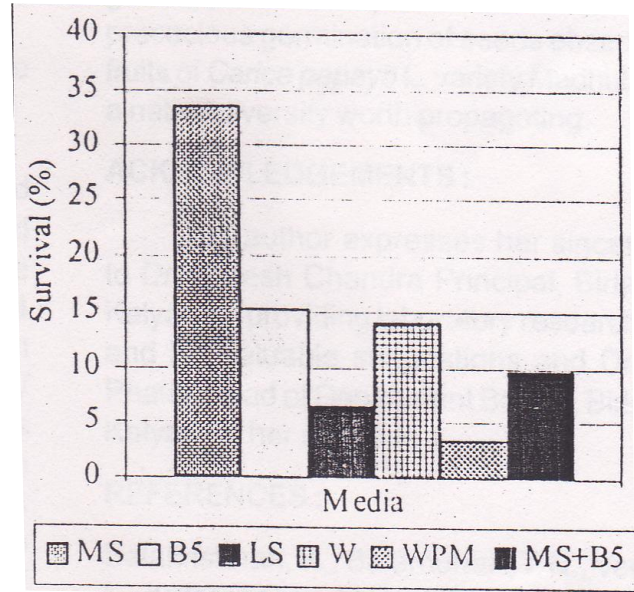


Fig. 3 : Survival (%) of precociously germinated seedlings of *Carica papaya* L. variety Madhubindu on different media (regenerated in vitro)

Table 1 : Response of node and shoot apex explants in *Carica papaya* L. variety Madhubindu inoculated on MS medium fortified with BAP, AS and NAA in various concentration supplemented with 10% coconut milk.

MS medium	Coconut Milk	Carica papaya L. variety Madhubindu	
		Shoot bud numbers	
BAP + AS + NAA (Mg/l)		Node	Shoot apex
2.0 + 0.5 + 0.5	-	2.84 ± 0.24	2.90±0.21
2.0 + 0.5 + 1.0	-	2.26 ± 0.17	2.42±0.13
2.0 + 1.0 + 0.5	-	2.27 ± 0.16	2.39±0.16
2.0 + 1.0 + 1.0	-	1.91 ± 0.17	2.07±0.12
2.0 + 0.5 + 0.5	10%	3.41± 0.16	3.47±0.17
2.0 + 0.5 + 1.0	10%	2.22 ± 0.10	2.27±0.13

2.0 + 1.0 + 0.5	10%	2.38 ± 0.17	2.42±0.16
2.0 + 1.0 + 1.	10%	1.77 ± 0.14	1.78±0.11

Values are mean of three sets of determinats. Each set containing 10 explants.

BAP - 6 - Benzyl aminopurine ; AS - Adenine sulphate; NAA - Naphthalene aceticacid.

Node and shoot apex explants inoculated on MS lmedium fortified with auxins and cytokinins singly or in combination showed the establishment and initiation of shoot buds in 4 weeks. Node explants inoculated on MS medium fortified with BAP + AS + NAA (2.0 + 0.5 + 0.5 mg/l) and CM (10%) gave rise to 3.41 shoot buds (Fig. 1 D). Shoot apex explants inoculated on MS medium fortified with BAP + AS + NAA (2.0 + 0.5 + 0.5 mg/l) and CM (10%) gave rise to 3.47 shoot buds (Table 1). In vitro rooting of multiple shoots gave rise to plantlets which were transferred to soil : vermiculite :cowdung (1:1:1) for hardening and finally transferred to field. Fruits from tissue cultured plantlets showed vivipary in fruit though all seeds in a fruit did not show precocious germination.

Carica papaya L. is an economically important tropical plant grown mainly for its fruits. Diversity in papaya is well known, some diversities are well collected and others poorly represented. *Carica papay* L. variety Madhubindu showed precocious germination of seeds. Direct transfer of the precociously germinated seedlings on combination of soil: vermiculite: cowdung (1:1:1) showed poor response. The precociously germinated seedlings were inoculated on different media. It showed 33.6% survival on MS medium. For mass propagation different varieties of papaya have been successfully regenerated in vitro by nodal explants (Saha et. al., 2004), somatic embryogenesis (Saha et al., 2004), synthetic seeds (Saha et al. 2004). axillary bud explants (Saha et al. 2004). Node and shoot apex explants from precociously germinated seedlings of *Carica papaya* L. variety Madhubindu served as an important means for mass propagation. In vitro techniques have great potential for propagation and conservation of genetic resources of endangered species, elite genotypes and

genotypes with special attributes and the precocious germination of seeds observed in he fruits of *Carica papaya* L. variety Madhubindu was a native diversity worth propagating.

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