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Research Article

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In Vitro Regeneration of Papaya (*Carica papaya* L.) Variety Surya

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ABSTRACT

An efficient protocol was developed for micro-propagation of Indian papaya (Carica papaya L.) var. Surya. Shoot bud induction occurred on culturing immature zygotic embryos segments in MS (Murashige andSkoog, 1962) medium, followed by transfer onto shoot multiplication, elongation and rooting media. ATreatments with various growth regulators resulted in the production of callus initially followed by induction of shoot buds on subculture to the same medium. among the various growth regulators used viz., BAP, Kinetin, TDZ or 2, 4-D, Maximum per cent of (91.7) callus induction was observed with TDZ at 0.1 mg/l. GA_3 at al concentrations was found to induce elongation of shoot buds and maximum number of elongated shoots/ explants (14) was obtained at 1.0 mg/l and thereafter there was a decline in the number of elongated shoots/ explant. The same concentration was found to give rise to maximum extent of elongation with shoots measuring 3.0 cm. Rooting was observed only at 2.0 and 3.0 mg/l with 14.2 % and 28.4% rooting response. The in vitro-raised plantlets were successfully hardened first under culture room conditions, then in greenhouse with 70 % survival rate.

Key words: *Carica papaya, Immature zygotic embryos , Thidiazuron*

INTRODUCTION

Papaya (*Carica papaya* L.) is an economically important fruit crop belongs to the family Caricaceae. The crop was grown in the tropical and sub-tropical regions of the world. Major papaya producing countries are Brazil, Mexico, Nigeria, Indonesia, India, Ethiopia, Congo, Peru, China and the Philippines. A small dicotyledonous family consists of four genera. Carica papaya is the largest genus with 22 described species. There are three primary sex types which include pistillate, staminate and hermaphroditic type plants of indeterminate sex type. It is rich source of carbohydrates, minerals, vitamin A and C, pectin, alkaloid and carpine. Papaya is used as fresh, ripened fruit as well as raw vegetable. This fruit is gaining more importance due to extraction of latex which is known to contain an enzyme papain and chymopapain[1]. The papain is used in tenderizing of meat, manufacture of cosmetics, curing of leather, brewing, chill proofing of beer and treatment of digestive disorders.

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However its cultivation is hindered by problems due to the inherent heterozygosity and dioecious nature production of non-true-to-types seeds of open-pollinated flowers exhibit considerable variation in shape, size and flavour and susceptibility to papaya ring spot virus[7]. Papaya cultivation is mainly hampered by the presence of papaya ring spot virus and virtually no resistance has been found in cultivar available for conventional breeding. Introgression of resistance genes from wild species into commercial cultivar has been attempted through conventional breeding but disease resistant cultivars have not yet been produced. Hence the current importance of genetic transformation by uptake of exogenous DNA has generated enormous interest in harnessing the advantage offered by plant tissue culture and gene transfer technology .Regeneration of papaya plants has been reported from protoplast, cotyledon, petiole, hypocotyls, root, anther, ovule and immature zygotic embryo cultures significant progress has been achieved using organogenesis and somatic embryogenesis[3].

MATERIAL AND METHODS

Establishment of aseptic cultures

For initiation of cultures embryos derived from selfed immature fruits of carica papaya cultivar Surya were used as explants which are hybrid derivative of SUNRISE SOLO X PINK FLESH SWEET released from IIHR, Bangalore. It is gynodioecious smooth skinned flesh has attractive red colour, soft, crisp, free from typical papaya odour with high TSS. The fruit was first thoroughly washed in tap water for 2 to 3 times. The fruits were then surface sterilized with 70 per cent ethyl alcohol for 15 to 20 minutes followed by flaming under aseptic conditions. Under sterile conditions, fruits were cut open and ovules/ seeds were separated aseptically and stored in a sterile bottle under refrigerated conditions for further use.

Culture conditions

The various stock solutions and plant growth regulators were dispensed separately. The pH of the medium containing all the above components was adjusted to 5.8 using 0.1 N HCl or NaoH. Culture tubes containing the medium were steam sterilized in autoclave (pressure: 1.06 Kg/cm2; temperature 121^0C) for 15 to 20 minutes. After sterilization, the culture tubes with the prepared media were allowed to cool at room temperature. All the cultures were grown at 25±2°C under a 16-h photoperiod. The illumination was provided by cool white fluorescent tubes at a light intensity of 30-40 μ mol m^{-2} S^{-1} PAR.

EXPERIMENTAL RESULTS

In vitro regeneration

Embryo obtained from 120 days old papaya CV Surya were inoculated on medium supplemented with various growth regulators viz., Cytokinins such as TDZ,BAP, Kinetin or Auxins such as 2,4-D at concentrations of 0.05,0.1,0.25,0.50,1.0,1.5 and 2.0 mg/l. The embryos showed varied response in terms of callusing and shoot bud formation. Embryos inoculated on MS basal medium devoid of any plant growth regulators showed no response. There was callus induction from cultured embryos at all concentrations of cytokinins and 2,4-D tried. However, the percent response varied with the concentration of growth regulators used. Maximum per cent callusing response of (91.7%) was obtained with TDZ at 0.1 mg/l, beyond this level the per cent callusing started declining with every increment in TDZ level. In case of BAP, however callusing response increased with increasing concentration of BAP. The maximum per cent callusing (70.7%) was observed at 2.0mg/l of BAP, while in case of kinetin there was increasing in callusing RESPONSE (58.2%) up to 1.0mg/l beyond this level the per cent callus induction started declining with every increment in kinetin level. However, in medium supplemented with 2,4-D the per cent callusing response increased with increasing concentrations. Maximum callusing response (41.6%) was obtained at 2.0mg/l. The above results suggested that the cytokinins TDZ at 0.1 mg/l was best for inducing maximum callusing response. The callus on subculture to the medium gave rise to

the shoot buds after 3 weeks. Induction of shoot buds followed the same pattern as that of callus induction. The concentration of cytokinin, TDZ which gave rise to maximum per cent callusing was found to give rise to maximum shoot buds per explants (15.6) in BAP supplemented medium maximum of 10 shoot buds/ explant was obtained at 2.0mg/l BAP which also gave maximum per cent callusing response. Similarly maximum shoot buds per explant of 5.0 and 3.5 in kinetin and 2,4-D supplemented media at concentration of 1.0 mg/l kinetin and 2.0mg/l, 2,4-D respectively coincided with the maximum per cent callusing response in these two growth regulators (Table 1).

Table 1: Effect of Plant Growth Regulators (PGRS) on shoot bud regeneration response from immature zygotic embryos

Sl. No	PGR Treatment (mg/l)				% callus regeneration				No of shoot buds/explants			
	TDZ	BAP	Kinetin	2-4-D	TDZ	BAP	Kinetin	2-4-D	TDZ	BAP	Kinetin	2-4-D
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0.05	0.05	0.05	0.05	70.83	16.64	12.48	8.32	9.3	2.0	1.0	0
3	0.1	0.1	0.1	0.1	91.66	20.8	16.64	12.48	15.6	2.5	1.5	0
4	0.25	0.25	0.25	0.25	74.88	24.96	29.12	20.76	10.5	4.0	3.0	2
5	0.50	0.50	0.50	0.50	66.56	39.12	99.92	16.64	6.0	5.0	4.5	1.5
6	1.0	1.0	1.0	1.0	62.4	62.4	58.24	33.28	5.5	7.0	5.0	3.0
7	1.5	1.5	1.5	1.5	41.6	66.56	45.76	37.44	4.0	8.0	3.5	3.5
8	2.0	2.0	2.0	2.0	24.96	70.72	37.44	91.6	2.0	10.0	2.5	3.5

24 explants per each treatment with 3 replications Observations taken 5 weeks after incubation

Elongation of shoot bud

The explants with maximum number of shoot buds/ explants from TDZ medium at 0.1 mg/l concentration were cultured in medium supplemented with GA_3 at different concentration viz., 0.1,0.25,0.5,1.0,2.0 and 5.0 mg/l. GA_3 was found to be necessary for elongation of shoot buds because in its absence, the shoot buds did not elongate. GA_3 at all concentration was found to induce elongation of shoot buds and the number of elongated shoots per explants increased with increase in concentration of GA_3 used and maximum number of elongated shoots of 14 per explants was obtained at 1.0mg/l concentration. Thereafter the number of shoots per explants declined. The same concentration was suitable for giving rise to shoots with maximum length of 3.0cm. at higher concentration of GA_3 more than 1.0mg/l, there was less elongation of shoots (2.3 v/s 2.0 cm) but the length of shoots was better than that observed at lower concentration of GA_3 (0.4 to 1.6 cm) at 1.0 mg/l (table2).

Table 2: Effect of GA_3 on shoot elongation from regenerated cultures of immature zygotic embryos of papaya.cv. Surya.

SL.NO.	GA_3 (mg/l)	No of elongated shoots / explants	Shoot length (cm)
1	0.0	0	0
2	0.1	2	0.4
3	0.25	6	1.0
4	0.50	7	1.6
5	1.00	14	3.0
6	2.00	5	2.3
7	5.00	4	2.0

7 Treatments with 6 replications
Observations were taken after 4 weeks of incubation

Rooting

The elongated shoot lets were transferred to MS media supplemented with IAA,IBA and NAA at different concentration viz., 0.5,1.0,2.0,3.0 and 5.0 mg/l. Rooting was observed only in IAA medium at 2.0 and 3.0 mg/l with a response of (14.2 %) and (28.4%) respectively (Table3).

Table 3: Effect of IAA on rooting of shoot lets produced *in vitro*

SL. No	Concentration (mg/l)	No. of rooted shoots	% response
1	0.0	0.0	0.0
2	0.5	0.0	0.0
3	1.0	0.0	0.0
4	2.0	1.0	14.2
5	3.0	2.0	28.4
6	5.0	0	0.0

7 replications for every treatment
Number of days taken for rooting 30 days

DISCUSSION

Some studies on regeneration were carried out using papaya the first step in any plant cell or tissue culture system is to obtain regeneration using suitable explants. The type of the explants, its size, age and manner in which it is cultured can determine whether the culture can be successfully maintained and morphogenesis could be induced. Guha and Maheshwari were the first to show that embryo could be used as explants. Embryo cultures of polarized egg, zygote, pro embryo or mature embryo on a suitable medium for obtaining regeneration could be used. Usually embryo culture is followed to obtain rare interspecific hybrids as in case of papaya[4] and for haploid production (barley). To overcome dormancy and shortening the breeding cycle of deciduous trees (Musa sp) this technique can be used. Besides, this technique is also used for micropropagation and establishing regeneration system for genetic manipulations[5].

Regeneration response from immature embryos

In vitro regeneration was attempted using immature zygotic embryos as explants. Regeneration of plantlets have been achieved using almost all explants both vegetative such as hypocotyls, cotyledon, Petiole, stem, leaf, Protoplasts, root and reproductive explants such as anther, ovule and immature zygotic embryo[1]. However, they have found ovule and embryo tissues to be most ameanable for regeneration. The most preferred pathway of regeneration was found to be the somatic embryogenesis pathway in all these explants[4]. Even though regeneration through organogenesis has also been reported extensively[6]. In the present study regeneration has been achieved through indirect organogenesis mediated through a callus pathway. Treatments with various growth regulators resulted in the production of callus initially followed by induction of shoot buds on subculture to the same medium. among the various growth regulators used viz., BAP, Kinetin, TDZ or 2,4-D, Maximum per cent of (91.7) callus induction was observed with TDZ at 0.1 mg/l; beyond this level callusing per cent declined with every increment in TDZ level. In case of BAP maximum per cent callusing (70.7) was observed at 2.0 mg/l while in kinetin media maximum per cent callusing was observed at 1.0mg/l. Induction of shoot buds followed the same trend with maximum shoot buds/ explants of 15.6 as obtained in TDZ supplemented medium (0.1 mg/l). Similarly, maximum regeneration of shoot buds of 10 and 5 was obtained in BAP and Kinetin supplimented media of 2.0 mg/l BAP and 1.0 mg/l Kinetin respectively. Similar result at callus induction from leaf, petiole, stem and root explants has been

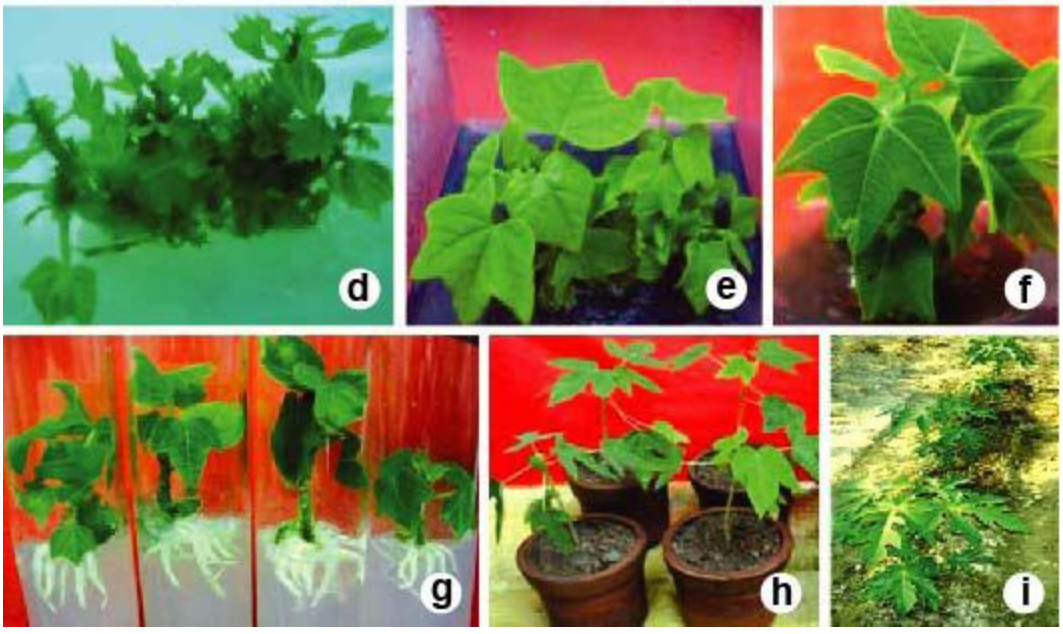
reported from media supplemented with BAP and IBA[9]. Induction of papaya callus from immature zygotic embryos on MS media supplemented with TDZ (0.1 TO 2.0 mg/l) has been reported by yang 2001. Generally 2,4-D has been used for elicitation of embryogenic response from somatic tissues.there is production of embryogenic callus initially after 3 months on induction medium supplemented with 2,4-D (2.0 TO 112.5µM) and development of somatic embryos directly thereafter from embryogenic callus on induction medium or more often they differentiate from callus subcultured on medium devoid of growth regulators.

Elongation of shoot buds

Explants with maximum shoot buds/ explants (15.6) were obtained in TDZ medium (0.1 mg/l) and those were transferred to medium supplemented with GA_3 at various concentrations ranging from (0 to 5.0 mg/l). Presence of $_{GA3}$ was found to be critical for induction of shoot elongation as the shoot buds fail to elongate in its absence. $_{GA3}$ at al concentrations was found to induce elongation of shoot buds and maximum number of elongated shoots/ explants (14) was obtained at 1.0 mg/l and thereafter there was a decline in the number of elongated shoots/ explants. The same concentration was found to give rise to maximum extent of elongation with shoots measuring 3.0 cm. Many workers could achieve shoot elongation on prolonged culture in the same rooting medium[3], although GA_3 (0.1 TO 3.0 mg/l) has been used for shoot elongation.

Rooting of shoots

In the present study, the elongated shoots were transferred to MS media supplemented with IAA at various concentrations. Rooting was observed only at 2.0 and 3.0 mg/l with 14.2 % and 28.4% rooting response. IBA has been most commonly used by several and combination of IAA (0.1 to 2.0mg/l) and IBA (0.5 to 5.0mg/l) has been used[9].



Hardening and acclimatization

In vitro regenerated plantlets were washed carefully in running tap water to remove the traces of agar. They were transferred to culture tubes containing quarter-strength liquid MS salts without sucrose for 24-48 h and then transferred to pots containing autoclaved soil and soil rite (1:1, w/w) and were covered with poly bags for 4 wk to maintain high RH. The plantlets were initially irrigated with quarter-strength inorganic salts of MS medium for 2 wk followed by tap water. Potted plantlets were grown in culture room conditions (25±2°C, 55±5%RH, under 16 h of photoperiod with a light intensity of 40 µ mol m-2 s-1) for 2months. Poly bags were removed

gradually upon emergence of new leaves and acclimatized plantlets were transferred to the greenhouse.

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