Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(3): 1954-1956
Received: 21-03-2019
Accepted: 25-04-2019

Suvalaxmi Palei
Department of Fruit Science and Horticultural Technology, Orissa University of Agriculture and Technology, Odisha, India

DK Dash
Department of Fruit Science and Horticultural Technology, Orissa University of Agriculture and Technology, Odisha, India

GR Rout
Department of Agricultural Biotechnology, Orissa University of Agriculture and Technology, Odisha, India

Correspondence
Suvalaxmi Palei
Department of Fruit Science and Horticultural Technology, Orissa University of Agriculture and Technology, Odisha, India

Standardization of *in vitro* protocol for plant regeneration of *Carica papaya* cv. Co8 through indirect organogenesis

Suvalaxmi Palei, DK Dash and GR Rout

Abstract
An efficient protocol was developed for micro- propagation of Indian papaya cv. CO8. Induction of callus by culturing apical buds were obtained in MS medium followed by transfer onto shoot multiplication, elongation and rooting media. The treatments with various growth regulators resulted in production of callus initially followed by induction of shoot buds on subculture to the same medium. Among the various growth regulators used like BAP, Kinetin, TDZ, 2,4-D. Maximum percent callus induction was observed with TDZ at 0.25 mg/l, GA_3 at 1.0 mg/l was found to induce maximum elongation of shoot buds and maximum number of elongated shoots per explants was obtained the same treatments of GA_3. Rooting was observed at 2.5 mg/l IBA supplemented in ½ MS media. The in vitro raised plantlets were successfully hardened under culture room having controlled atmospheric condition, then these were transferred to greenhouse with a survival rate of 81%.

Keywords: *Carica papaya*, callus, regeneration

Introduction
Papaya (*Carica papaya* L.) is an economically important fruit crop belongs to family caricaceae. The crop was grown in the tropical and subtropical climate throughout the world. Major papaya producing countries are Brazil, Mexico, Nigeria, Indonesia, India, Ethiopia, Congo, Peru, china, Philippines. Caricaceae is a small dicotyledonous polygamous family consist of four genera. *Carica is* the largest genus with 21 described species (Badillo, 1967) [1]. Plants of *Carica papaya* are small, herbaceous, evergreen, dicotyledonous fragile tree with a hollow, soft wooded 1 to 10 m high perennial which may produce fruit more than 20 years but economical life is not more than 3 years. Oschae *et al*. 1975 [6] classified papaya flowers and its plants into 5 groups like pistillate, pentandria, intermediate, hermaphrodite and staminate. Fruit is rich in Vitamin A, C, pectin, alkaloid and carpine and used as ripe fruit as well as for raw vegetables. Industrial value of papaya is increasing day by day due to the digestive enzyme papain. However its cultivation is hindered due to inherent heterozygosity and dioeceous nature production of non-true to the type seeds of open pollinated flowers which exhibit considerable variation in shape, size and flavor and susceptibility to papaya ring spot virus. Moreover, as sex cannot be determined until the mid-development stage, three seedlings are established in each planting position, till flowering. Then they are thinned, retaining only the most vigorous female plant with one male to every 10 to 20 female plants. This results in wastage of inputs. With a requirement to renew plantations every three year to ensure quality fruit production propagation by seed represents a significant cost to the producer. The multiplication rates are low in the vegetative propagation methods like mound layering. This field level problem necessitates the substitution of seedling progeny with tissue culture propagules developed from female or bisexual plants. Micro propagation represents the only economic way of continuously producing uniform planting materials of known sex. Papaya clones developed by *in vitro* method are uniform and produce high quality fruits similar to those of their mother plants (Chan and Teo, 2002) [4].

Materials and Methods
Establishment of aseptic culture
For initiation of culture meristem tip of young seedlings were collected and seedlings were raised under aseptic condition. The explants were then washed in running water and surface sterilized with $HgCl_2$ @ 0.01% for 10 minutes. After surface sterilization the explants were washed for 3 times in distilled water to remove extra $HgCl_2$ and then cultured in desired media.

Culture conditions

The various stock solutions and plant growth regulators were dispensed separately in MS media (Murashige and Skoog, 1962) [5]. The pH of the medium containing all the above components was adjusted to 5.8 using 0.1N HCl or NaOH. Culture tubes contain the medium were steam sterilized in autoclave (pressure: 1.06 Kg/cm^2; temperature 121 ^{0}C) 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.

Results and Discussion

***In vitro* regeneration**

Meristem tip obtained from 21 days old papaya seedlings were inoculated on medium supplemented with various growth regulators viz. TDZ, 2,4-D, BAP and Kinetin at concentration of 0.10, 0.25, 0.50, 1.0,1.25,1.50, 2.0 mg/l. The explants showed varied response in terms of callusing and shoot bud formation. Explants inoculated on MS basal medium devoid of any plant growth regulators showed no response. There was callus induction from cultured meristem tips at all concentration of 2, 4- D tried. However, the percent response varied with the concentration of growth regulators used. Maximum percent callusing response of (92.8%) was obtained with TDZ at 0.25 mg/l, beyond this level the percent callusing started declining with every increment in TDZ level. In case of BAP, however callusing response increased with increasing concentration of BAP. The maximum percent callusing 76.23% was observed at 2.0mg/l of BAP, while in case of Kinetin there was increasing response but 65.32% up to 1.0 mg/l beyond this level, percent callus induction started declining with increment in kinetin level. However, in medium supplemented with 2,4-D the per cent callusing response increased with increasing concentrations. Maximum callusing response (91.98%) was obtained at 2.0mg/l. The above results suggested that TDZ at 0.2 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 (9.86), in BAP supplemented medium maximum of 9.67 numbers of 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 3.91 and 3.33 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.

In vitro regeneration was attempted using meristem tip 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 embryo1. However, they have found meristem tissues and apical bud to be most amenable for regeneration. Even though regeneration through organogenesis has also been reported extensively (Panjaitan *et al*., 2007) [7]. 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. Similar result at callus induction from leaf, petiole, stem and root explants has been reported from media supplemented with BAP and IBA [Yang *et al*., 1996] [10]. 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 (Ramesh *et al.*, 2018). 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 sub-cultured on medium devoid of growth regulators (Bindu, 2015) [3]

Elongation of shoot bud

The explants with maximum number of shoot buds/ explants from TDZ medium at 0.25 mg/l concentration were cultured in medium supplemented with GA_3 at different concentration viz., 0.10,0.25,0.50,1.00,1.50,2.00 and 2.50 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 9.43 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 3.42cm. At higher concentration of GA_3 more than 1.0mg/l, there was less elongation of shoots but the length of shoots was better than that observed at lower concentration of GA_3. Presence of GA_3 was found to be critical for induction of shoot elongation as the shoot buds fail to elongate in its absence. Many workers could achieve shoot elongation on prolonged culture in the same rooting medium [Bhatacharya *et al*., 2003] [2], although GA_3 (0.1 to 3.0 mg/l) has been used for shoot elongation (Suthamathi *et al*., 2002) [9].

Rooting of micro shoots

The elongated shoot lets were transferred to MS media supplemented with IBA at different concentration viz., 0.5,1.0,1.5,2.0,2.5,3.0 and 3.5 mg/l. Rooting was observed in IBA medium at 2.0 to 3.5 mg/l with a response of (18.45 %) and (22.19%) respectively. 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 [Yang *et al*., 1996] [10].

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 weeks to maintain high relative humidity. The plantlets were initially irrigated with quarter strength inorganic salts of MS medium for 2 weeks 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 for 2months. In vitro raised plantlets were removed gradually upon emergence of new leaves and acclimatized plantlets were transferred to the greenhouse.

Table 1: Effect of plant growth regulators on callusing and shoot bud regeneration of *Carica papaya* cv. CO8

Sl. No.	PGR treatments (mg/l)				% callus regeneration				Number 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	0.10	0.10	0.10	0.10	68.89cb	19.43g	11.34e	11.21fe	5.32d	1.56g	1.32d	0
3	0.25	0.25	0.25	0.25	92.80a	21.67f	12.87e	18.20e	9.86a	2.98f	1.43d	0
4	0.50	0.50	0.50	0.50	71.56b	29.41e	29.00d	33.21d	8.67b	3.89e	2.67c	0
5	1.00	1.00	1.00	1.00	66.23cb	38.60d	65.32a	35.31d	8.45b	4.32d	3.91a	0
6	1.25	1.25	1.25	1.25	54.97d	62.17c	54.87b	45.20c	6.09c	6.54c	2.98c	1.98c
7	1.50	1.50	1.50	1.50	46.21e	69.70b	45.32c	56.21b	5.32d	7.65b	3.34ba	3.21ba
8	2.00	2.00	2.00	2.00	25.20f	76.23a	29.60d	91.98a	4.09e	9.67a	3.76a	3.33a

Table 2: Effect of GA_3 on shoot elongation from regenerated cultures of *Carica papaya* cv. CO8

Sl. No.	GA_3 mg/l	Numbers of elongated shoots per explants	Shoot length (cm)
1	0.0	0	0
2	0.10	2.45f	0.87e
3	0.25	5.43e	1.01d
4	0.50	6.98d	1.29d
5	1.00	9.43a	3.42a
6	1.50	8.38b	2.01b
7	2.00	7.21c	2.01b
8	2.50	5.30e	1.99cb

Table 3: Effect of IBA on rooting of shootlets produced *in vitro*

Sl. No.	Concentration mg/l	Number of rooted shoots	% response
1	0.00	0.00	0.00
2	0.50	0.00	0.00
3	1.00	0.00	0.00
4	1.50	0.00	0.00
5	2.00	2.98d	18.45d
6	2.50	4.75a	36.41a
7	3.00	3.76b	23.56b
8	3.50	3.10c	22.19c

Conclusion

Papaya is an important cash fruit crop owing to its first maturation rate and continuous fruit production in subtropical and tropical climate. There are reports on micropropagation of papaya from mature field grown trees. However, the technique could not be reproduced for mass cloning of papaya at commercial scale due to high contamination and latex oozing. The apical buds are proved to be best explant to regenerate plantlet through indirect organogenesis and can solve the problem to getting quality planting material free from virus.

References

1. Badillo VM. Esquem de la Caricaceae. Agronomid Tropical. 1967; 27:245-273.
2. Bhattacharya J, Khuspe SS, Renukdas NN, Rawal SK. Somatic embryogenesis and plant regeneration from immature embryo explants of papaya cv. Washington and Honey Dew. Indian J Exp. Biol. 2003; 40:624-627.
3. Bindu B. Micropropagation of Papaya variety CO-5, International journal of research in Agriculture and Forestry. 2015; 2(2):46-49.
4. Chan LK, Teo KH. Micro propagation of Eksotike, a Malaysian papaya cultivar and the field performance of the tissue culture derived clones. Acta Hort. 2002; 575:99-105.
5. Murashige T, Skoog FA. revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 1962; 15:473-97.
6. Ochase JJ, Jones Soule, Dighumar MJ, Wellhourg C. Papaya. Tropical and Subtropical agriculture, The Macmillan Co- collier Macmillan Ltd, 1975, 586-595.
7. Panjaitan SB, Aziz MA, Rashid AA, Saleh NM. In-Vitro Plantlet Regeneration from Shoot Tip of Field grown Hermaphrodite Papaya (*Carica papaya* L. cv. Eksotika). Int. J Agric. Biology. 2007; 6:827-832.
8. Ramesh AN, Santhosh DB, Suresh D, Radha Krishna, Rashmi K, Hajira Khanm. In vitro regeneration of papaya (*Carica papaya* L.) variety Surya; International journal of pure and applied bioscience. 2018; 6(4):456-461.
9. Suthamathi SJ, Haripriya K, Kamalakannan S. Micropropagation in papaya cv. CO-5.2002. Indian J. Hort. 2002; 59:13-16.
10. Yang J, Yu T, Cheng Y. Transgenic papaya plants from Agrobacterium mediated transformation of petioles of in vitro propagated multishoots. Plant Cell Rep. 1996; 15:459-464.