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STANDARDIZED *IN VITRO* PROTOCOL FOR REGENERATION AND HARDENING OF PAPAYA VAR. RED LADY USING PGRS

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ABSTRACT

Papaya is considered one of the important fruit crops and yields throughout the year. Micropropagation represents the economic way of continuously producing uniform and disease-free planting material. The present investigation entitled "Standardized in vitro Protocol for Regeneration and hardening of Papaya var. Red Lady Using PGRs" were carried out in the biotechnology laboratory and experimental field of Horticulture, faculty of agriculture, Guru Kashi University, Talwandi Sabo. BAP 2.50mg/l + IBA 0.2mg/l took minimum number of days for shoot initiation and produced highest number shoots, leaves and longest shoot length. IBA 2.00mg/l + NAA 2.00mg/l produced maximum rooting percentage, highest number of roots, shoots and leaves, longest shoot length and took minimum days for root initiation. Potting mixture containing a composition of Sand: Soil: FYM (1: 1: 1 v/v/v) gave maximum survival percentage, maximum height and leaves.
Keywords **:** Papaya, PGR's, MS media, Explant, Regeneration, Hardening.

Introduction

The papaya (*Carica papaya* L.) is a native of tropical America and belongs to family Caricaceae. It is a major tropical and subtropical fruit crop. India is the largest producer of papaya in the world with total production, 5744 thousand metric tonnes from an area of 149 thousand hectares. (NHB, 2022). In India papaya is mostly cultivated in the states of Andhra Pradesh (1,503.18 tonnes), Gujarat (1,107.88 tonnes), Maharashtra (496.12 tonnes), Karnataka (491.95 tonnes), Madhya Pradesh (489.08 tonnes), Chhattisgarh (379.56 tonnes), West Bengal (299.79 tonnes), Jharkhand (178.88 tonnes), Assam (152.72 tonnes), Telangana (122.51 tonnes). (NHB, 2021-22). In subtropics like Punjab the area under papaya cultivation were (7.01 hectare) with (136.62 MT) annual production in year (2020-2021) (HSD, 2021).

The nutritional and medicinal benefits of papaya are well established. The papaya fruit is grown for its great nutritional content and deliciousness. Protein, fat, fibre, carbs, minerals (potassium, phosphorus, and calcium), and vitamins (carotene, thiamine, riboflavin, ascorbic acid, and volatile compounds) are all abundant in it. Papaya latex, which is primarily extracted from the immature green fruits, includes the proteolytic enzymes papain and chymopapain. Papain, an enzyme found in papaya, may help break down proteins. The papain is used for tenderizing the meat, manufacturing of cosmetic products, curing of leather, brewing, chill proofing of beer and also used as treatment for digestive disorder (Ramesh *et al.*, 2018). The papaya is a herbaceous perennial plant that can reach a height of 10 meters and has a lifespan of less than 20 years (Hernandez Salinas *et al.*, 2022).

Essentially papaya is a cross-pollinated crop and propagated through the seed. Asexual methods such as cutting and grafting can also be used to create papaya plants, but because the mother plant only produces a small number of plants, these techniques are frequently time-consuming and unfeasible when used on a large scale (Setargie *et al.*, 2015). Micropropagation on papaya would aid in commercial production and solve a number of seedling-related issues. Through the use of in vitro cultures, papaya clonal propagation is now feasible. The study of micropropagation by tissue culture on papaya offer advantages over conventional propagation methods and aims at growing high quality fruits that has been widely applied to papaya species. A study on papaya (*Carica papaya* L.) cv. Surya micropropagation was conducted by Ryavalad *et al.*, (2019) for culture establishment, BAP (1-2 mg/l), NAA (0.5-0.1 mg/l), and Kinetin (0.5-1.0 mg/l) were utilised either separately or in combination. MS medium supplemented with BAP 2.00 mg/l was noticeably better than other treatments for culture establishment.

The present studies were focused to develop an efficient protocol for micropropagation under Punjab Sub-tropics with main objective for standardization in vitro shoot proliferation, rooting and hardening.

Materials and Methods

The present investigation entitled "Standardized in vitro Protocol for Regeneration and hardening of Papaya var. Red Lady Using PGRs" were conducted in the Biotechnology laboratory and experimental field of horticulture, university college of agriculture, Guru Kashi University, Talwandi Sabo. Nodal segment from mature plant were used as explant. For surface sterilization explants were rinsed off properly with tap water to remove soil and washed with 2-3 drops of labolene for 7-10 minutes. Then Bavistin (0.75%), streptomycin (0.1%) for 30 minutes and mercuric chloride (0.1%) for 3 minutes were used during experiment. The explants were inoculated on Murashige sand Skoog (MS) media with pH of the media was kept at 5.7 and the segments of sterilized nodal segments were removed by scalpel and forceps under laminar air flow chamber. The cultured bottles with explants were incubated at temperature of 25±2°C, 65 to 75% relative humidity, with photoperiod 16 hours of light and 8 hours of light with 3000 lux light intensity using white fluorescent light.

Expants were given different treatments at different concentration for shoot initiation and multiplication, rooting and hardening. In order to standardize best combination of growth hormones, different growth regulators BAP, IBA, NAA were used. The details of treatment are as:

Treatment Details

Table 1: Standardization of shoot proliferation

Treatment No.	Treatment
T_1	MS Media + BAP 1.0 mg/l
T_2	MS Media + BAP 1.5 mg/l
T_3	MS Media + BAP 2.0mg/l
T_4	MS Media + BAP 0.5mg/l + IBA 0.2mg/l
T_5	MS Media + BAP 1.0 mg/l + IBA 0.2mg/l
T_6	MS Media + BAP 1.5mg/l + IBA 0.2mg/l
T_7	MS Media + BAP 2.0 mg/l + IBA 0.2mg/l
T_8	MS Media + BAP 2.5 mg/l + IBA 0.2mg/l
T_9	MS Media + BAP 3.0mg/l + IBA 0.2mg/l

Table 2: Standardization of *in vitro* rooting

Treatment No.	Treatment
F_1	MS Media (control)
F_2	MS Media + IBA 1.5mg/l
F_3	MS Media + IBA 2.5mg/l
F_4	MS Media + NAA 1.5mg/l
F_5	MS Media + NAA 2.5mg/l
F_6	MS Media + IBA 1.5mg/l + NAA 1.5mg/l
F_7	MS Media + IBA 1.5mg/l + NAA 2.5mg /l
F_8	MS Media + IBA 2.5mg/l + NAA 1.5mg/l
F_9	MS Media + IBA 2.0mg/l + NAA 2.0mg/l
F_{10}	MS Media + IBA 2.5mg/l + NAA 2.5mg/l

Table 3: Effect of hardening treatments on survival and growth of *in-vitro* produced papaya plantlets

Treatments	Details
H_1	Sand (100%)
H_2	Soil (100%)
H_3	Sand + Vermiculite (2:1v/v)
H_4	Sand + Soil + Vermiculite (1:1:1: v/v/v)
H_5	Sand + Soil + FYM (1:1:1 v/v/v)

Statistical Analysis

The collected raw data during experiment trial was transferred on excel sheet in Microsoft Excel. The experimental design used was CRD (completely randomized design) with four replications of each treatment. The data was analysed using the software OPSTAT developed by CCSHAU, Hisar.

Result and Discussion

Standardization of shoot proliferation

After the incubation of three weeks, minimum number of days taken for shoot initiation was (18.00 days), highest number of shoots (3.25), longest shoot length (2.25cm), highest number of leaves per culture (2.75) were recorded in treatment which contained MS medium fortified with BAP 2.5mg/l and IBA 0.2mg/l. Maximum days (37.50 days), minimum number of

shoots (0.25), shortest shoot length (0.25cm), lowest number of leaves (0.00) were observed in MS media supplemented with BAP 1.0mg/l. Nguyen *et al.,* (2018) also observed that the combination of cytokinin and auxin regenerated maximum number of shoots in papaya as compare to the effect of cytokinin only.

Standardization of in vitro rooting

Out of 10 rooting treatments, rooting treatments that took minimum number of days for root initiation (25.00 days), maximum rooting percentages (86.00%), highest number of roots (5.50) and longest root length (4.50cm), longest shoot (5.50cm) and maximum number of leaves (4.00) were observed in MS medium supplemented with IBA 2.00mg/l and NAA 2.00mg/l. Maximum time taken as compared with other treatments for root induction (36.00 days), minimum rooting percentage (11.50%), minimum number of roots (1.75), the shortest root length (1.25cm), Smallest shoot (2.00cm), minimum number leaves (1.00) were found in treatments that contained MS basal medium.

Similar results were obtained from Patel *et al*., (2013), Samanmalie *et al.,* (2017), Nguyen *et al.,* (2018) and Palei *et al.,* (2019), in papaya.

Effect of hardening treatments on survival and growth of in-vitro produced papaya plantlets

Potting mixture containing a composition of Sand: Soil: FYM (1: 1: 1 v/v/v) gave maximum survival percentage (72.00%), maximum height (7.25) and maximum number of leaves (5.00). The lowest survival rate was observed in (5.75%) and minimum number of leaves (2.25) in soil were used 100%. The smallest height (4.25cm) were observed in both sand and soil when using alone at 100%.

Reports of Babu *et al.,* 2002 supports the result as they also obtained better survival and growth of in vitro plantlets in the potting mixture containing composition of soil: sand: FYM (1:1:1) in papaya plantlets.

Lalrinsanga *et al*., 2013 also supported the result that shown the survival of plantlets were best in sand: soil: FYM (1:1:1 v/v/v).

Conclusion

The higher proliferation of shoots with optimum number and length was achieved when the explants were cultured in MS media consisting of BAP 2.50mg/l with IBA 0.2mg/l. Early rooting with highest rooting percentage and optimum root length were obtained when *in vitro* regenerated shoots were cultured on MS media fortified with IBA 2.0mg/l and NAA 2.0mg/l. The rooting percentage were better when IBA and NAA were added in combination rather than singly. During hardening the potting mixture containing a composition of Sand: Soil: FYM (1:1:1 v: v: v) gave maximum survival percentage with highest number of leaves and optimum height.

Table 4: Effect of growth hormones on shoot proliferation

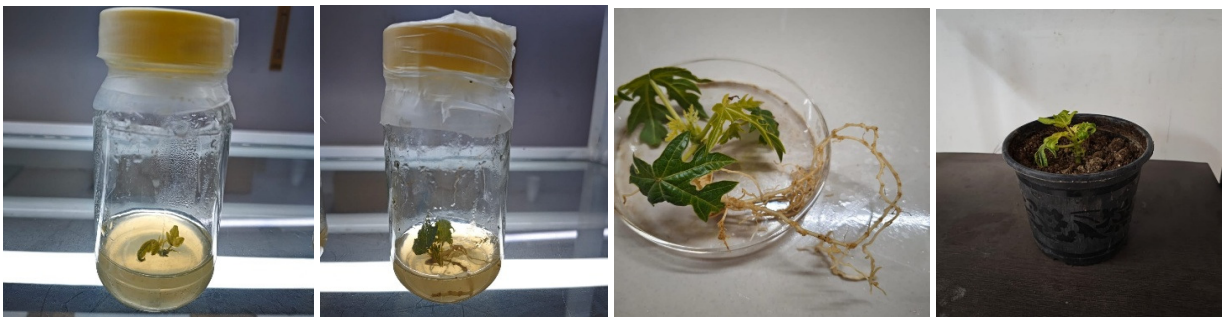
Treatment No.	Treatments	No. of days for shoot initiation	No. of shoots per culture	Length of shoot (cm)	No. of leaves per culture
T_1	MS Media + BAP 1.0mg/l	37.50	0.25	0.25	0.00
T_2	MS Media + BAP 1.50mg/l	31.25	0.50	0.50	0.25
T_3	MS Media + BAP 2.00mg/l	31.00	1.50	0.50	0.50
T_4	MS Media + BAP 0.5mg/l + IBA 0.2mg/l	30.25	1.50	0.75	1.00
T_5	MS Media + BAP 1.0mg/l + IBA 0.2mg/l	28.50	1.25	1.00	1.25
T_6	MS Media + BAP 1.5mg/l + IBA 0.2mg/l	25.50	1.75	1.25	2.00
T_7	MS Media + BAP 2.0mg/l + IBA 0.2mg/l	19.00	2.25	2.00	2.50
T_8	MS Media + BAP 2.5mg/l + IBA 0.2mg/l	18.00	3.25	2.25	2.75
T_9	MS Media + BAP 3.0mg/L + 0.2mg/l	21.75	2.00	1.75	2.25
	C.D (0.05)	0.64	0.76	0.67	0.62
	SE(m)±	0.22	0.26	0.23	0.21
	SE(d)	0.31	0.37	0.32	0.30
	C.V(%)	1.63	33.88	40.52	30.98

Table 5: Effect of growth hormones on in vitro rooting

Treatment No.	Treatments	Time taken for root initiation (days)	Culture rooting (%)	No. of roots per culture	Length of root (cm)	Length of shoot (cm)	No. of leaves per shoot
F_1	MS Media (control)	36.00	11.50	1.75	1.25	2.00	1.00
F_2	MS Media + IBA 1.5mg/l	34.00	16.50	2.00	1.75	2.25	1.75
F_3	MS Media + IBA 2.5mg/l	33.25	18.25	2.25	2.00	2.50	2.00
F_4	MS Media + NAA 1.5mg/l	32.25	22.50	2.75	2.50	2.75	2.25
F_5	MS Media + NAA 2.5mg/l	31.50	36.75	3.00	2.75	3.25	2.75
F_6	MS Media + IBA 1.5mg/l + NAA 1.5mg/L	30.00	40.50	3.25	3.00	3.50	3.00
F_7	MS Media + IBA 1.5mg/l + NAA 2.5mg/l	28.25	52.25	3.50	3.25	4.00	3.25
F_8	MS Media + IBA 2.5mg/l + NAA 1.5mg/l	28.00	58.25	4.50	3.50	4.25	3.50
F_9	MS Media + IBA 2.0mg/l + NAA 2.0mg/l	25.00	86.00	5.50	4.50	5.50	4.00
F_{10}	MS Media + IBA 2.5mg/l + NAA 2.5mg/l	27.25	68.50	4.75	4.25	5.00	3.75
	C.D (0.05)	0.53	0.74	0.68	0.68	0.64	0.57
	SE(m)±	0.18	0.25	0.23	0.23	0.22	0.19
	SE(d)	0.25	0.36	0.33	0.35	0.31	0.28
	C.V(%)	1.19	1.25	14.26	16.49	12.77	14.60

Table 6: Influence of potting mixture on survival and growth of plantlets

Treatment No.	Treatments	Survival (%)	Height (cm)	No. of leaves per plantlet
H_1	Sand (100%)	10.50	4.25	3.25
H_2	Soil (100%)	5.750	4.25	2.25
H_3	Sand + Vermiculite (2:1v/v)	52.00	5.00	4.25
H_4	Sand + Soil + FYM (1:1:1: v/v/v)	72.00	7.25	5.00
H_5	Sand + Soil + Vermiculite (1:1:1 v/v/v)	69.75	6.25	4.50
	C.D (0.05)	0.62	0.68	0.70
	SE(m)±	0.20	0.22	0.23
	SE(d)	0.28	0.31	0.32
	C.V(%)	0.97	8.28	12.09



(a) **(b)** **(c)** **(d)**

Fig. 1: (a) Shoot proliferation, **(b)** *In vitro* rooting, **(c)** Rooting and **(d)** Hardening of Papaya

Author Disclaimer

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during writing or editing of this manuscript.

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