

FACTORS AFFECTING IN VITRO ESTABLISHMENT AND GROWTH OF PAPAYA (*Carica papaya* L.) VAR. RED LADY

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ABSTRACT

Despite of desirable characteristics exhibited in Red Lady variety of papaya, growers are not able to adopt this variety due to very high cost of seed. Papaya, cross pollinated crop, commonly propagated through seed. Hence, importance of the problem is evident in lack of true-to-type. The technique of in vitro culture has been made clonal propagation a possibility in papaya. While standardizing the method of micropropagation of papaya, the factors influencing in vitro establishment and growth of papaya were examined namely, sources of explants, surface sterilants, establishment of explants and serial subcultures. Result revealed that higher rate of establishment was observed in shoot tip culture as compared to axillary bud. Surface sterilization treatments showed better response with $HgCl_2$ (0.1%) for 3 minutes in shoot tip explants collected from young seedling plants, while, in case of axillary bud explants collected from mature plants response with $HgCl_2$ (0.1%) for 10 minutes. Maximum establishment of explants (85.00%) was recorded in treatment MS + 0.5 mg/l BAP and 0.1 mg/l NAA.

KEY WORDS: *Explants, in vitro, micropropagation, papaya*

INTRODUCTION

Papaya (*Carica papaya* L.) belongs to family Caricaceae. Papaya is a native of tropical of North and South America (Litz, 1984). A ripe fruit of papaya is an excellent breakfast or dessert delicacy and the edible portion of fruit consists of carbohydrates (12.8%), rich source of ascorbic acid and provitamin 'A' (Chen and Tang, 1979). Production is also increased by adopting high yielding variety of papaya. Recently, in Gujarat var. Red Lady of papaya is being cultivated on large scale due to early mature, vigorous, high productivity with good keeping quality of fruit and tolerant to papaya ring spot virus. Although, desirable characteristics possessed by variety Red Lady, the growers

are not able to adopt this variety due to very high cost of seed. Papaya is propagated through seed. It is a cross-pollinated crop, hence, lack of true-to-type plant. Clonal propagation is an urgent necessity for improvement of papaya. The technique of *in vitro* cultures has been made clonal propagation a possibility in papaya. Litz and Conover (1978) and Rajeevan and Pandey (1983) has demonstrated the feasibility of multiplication of papaya through tissue culture. Hence, the experiment was planned with a view to examine the influence of the different factors on in vitro establishment and growth of papaya var. Red Lady for standardizing the protocol of micropropagation.

MATERIALS AND METHODS

Present investigation on “Micropropagation in papaya var. Red Lady” was carried out at Tissue Culture Laboratory, Department of Biotechnology, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari. While standardizing the methods for micropropagation in papaya, different factors influencing *in vitro* establishment and growth of papaya were studied i.e. sources of explants (shoot tip and axillary bud), surface sterilant (HgCl₂: 0.05% each for 5 and 10 min; 0.1% each for 3 and 5 min and 0.2 % for 2 min dip). The experiment was conducted with two different sources of explant materials. Out of which shoot tip explants collected from 4-6 weeks old nursery-raised papaya plants var. Red Lady grown at Horticulture Nursery, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari, while another source of explants i.e. axillary bud collected from mature plant var. Red Lady (approximately 7-8 months old plant) grown at Soil and Water Management Unit Farm, Navsari Agricultural University, Navsari, were used as mother (donor) plants. These explants were swabbed with cotton dipped in 70 per cent absolute alcohol and washed thoroughly in running tap water for 2-3 hours to remove traces of alcohol, dirt and latex. The explants were then, kept in a solution of 0.05 per cent bavistin (carbendazim 50 per cent WP) and 0.01 per cent streptomycin for two hours, followed by treatment with 10 per cent solution of detergent (Teepol) for 10 minutes. All traces of detergent were removed by repeated washing the explants thoroughly with double distilled water. The size of sterilized explants was further reduced to 0.8 cm in length. The trimmed explants were quickly inoculated on nutrient MS medium (Murashige and Skoog, 1962) supplemented with 0.5 mg/l BAP + 0.1 mg/l NAA + 30 g/l sucrose and 8 g/l agar for all factors. The cultures were incubated at 26±2° C in culture room. The

culture vessels and media were autoclaved at pressure of 15 lb/ inch² for 20 minutes at approximately 121°C temperature. After which autoclaved media were transferred to an air conditioned room for storage before use.

RESULTS AND DISCUSSION

Papaya is one of the few fruiting plants of commercial value to be propagated by *in vitro* micropropagation. Micropropagation requires the organs and tissues to be passed through a sequence of steps in which different cultural and environmental conditions are provided. The stage I being physiological preconditioning of explant and culture establishment, while stage II is being rapid multiplication of shoots.

Surface sterilization of treatments for papaya explants var. Red Lady

It is apparently seen from Table 1 and Plate 1 that establishment, contamination, death of culture and growth of explants from shoot tip was significantly influenced by sterilization treatments. Maximum establishment of explants (83.33 %) was recorded in treatment HgCl₂ (0.1 %) for 3 minutes dip (T₃) followed by T₄ (35.00 %), T₅ (25.00 %), T₁ (21.00 %), T₂ (10.00 %) and T₆ (6.00 %) (Fig. 1). Although, contamination was controlled maximum in T₄ (8.33 %) followed by T₅ (10.00 %), T₆ (10.00 %) and T₃ (12.00 %). Growth of shoot was suppressed as increased the duration of treatments. Maximum length of shoot (1.10 cm) was reported in treatment T₃ followed by T₄ (0.90 cm) and T₅ (0.80 cm), indicated that shoot length was higher at lower concentration and shorter duration of surface sterilizer used. It is also seen from Table 2 that establishment, contamination, death of culture and growth of explants from axillary buds was significantly influenced by sterilization treatments. Maximum establishment of explants (12.17 %) was recorded in treatment HgCl₂ (0.1 %) for 10 minutes dip (T₅) followed by T₃ (10.00 %). Although, contamination percentage were more than 54.00 in all the treatments.

Maximum length of shoot (1.10 cm) was reported in treatment T₅ follow by T₁ (0.93 cm) and T₄ (0.80 cm). Hence, it was revealed that explants collected from shoot tip, HgCl₂ (0.1 %) for 3 minutes and for axillary bud HgCl₂ (0.1 %) for 10 minutes were found to be very effective for sterilization and survival of explants. Naik (1997) was also able to sterilize effectively papaya cv. Honey Dew explants shoot tip of seedling (4-6 weeks) and mature female plants (7-8 months old) using 0.1 per cent HgCl₂ for 5 minutes and 10 minutes, respectively. Similarly, Rajeevan and Pandey (1983) were reported satisfactorily sterilization of papaya explants using 0.1 per cent HgCl₂ for 5 to 15 minutes. The intensity of contamination might be more in mature explants as they were collected from field grown plants, which might be due to the endogenous microbial infection which is less in seedling explants (Litz and Conover, 1981; De Winnaar, 1988; Agnihotri *et al.*, 2004).

Effect of plant growth regulators on establishment of shoot tip explant of papaya var. Red Lady:

For standardizing a suitable establishment medium for shoot tip explants, a trial was conducted using MS medium with 18 treatments combinations using different level of plant growth regulators (NAA, BAP and Kinetin). The results are presented in Table 3 and illustrated in Plate II and Fig. 3. Maximum establishment of explants (85.00 %) was recorded in treatment MS + 0.5 mg/l BAP and 0.1 mg/l NAA (E₁) followed by treatment E₄ (80.00 %), E₇ (80.00 %), E₈ (69.67 %) and E₂ (67.00 %). Further, it was seen that lower levels of BAP and NAA concentrations were more responsive for establishment of explants. The establishment of explants was reduced as increased BAP level in the medium. Similarly a minimum day taken for establishment was observed at lower level of plant growth regulators. i.e. MS medium supplemented with 0.5 mg/l BAP + 0.1 mg/l NAA (E₁) (4.00 day) followed by E₇ (5.33 day), E₁₀ (5.50), and E₂

(5.67 day). The trend for response of treatments was decreased as increased the levels of BAP, NAA and Kinetin in the medium. Maximum length of shoot (1.30 cm) was registered in treatment MS medium supplemented with 0.5 mg/l BAP + 0.1 mg/l NAA (E₁) followed by E₁₇ (1.20 cm) and E₁₀ (1.02 cm). Overall, treatment E₁ was found the most effective among all the treatments.

In the present investigation, establishment of axillary bud of female mature plant was greatly hampered due to leaching of latex released from the cut end of the explants, which form a thin layer at the cut end of the explants, thereby checking the movement of chemical from medium to the tissue, resulting in death of tissue. Similar, finding was also reported by Arora and Singh (1978) and Naik (1997). Such, problem was not noticed in case of seedling explants. Further, axillary buds from female mature plant almost failed to establish in medium, as they were found to be less amenable for *in vitro* culture. Pandey *et al.* (1986) also reported that the establishment of lateral bud was very low.

Effect of serial sub-culturing on multiplication of shoot tip of papaya var. Red Lady

For standardizing a suitable multiplication medium and serial sub-culturing (up to 4 subcultures) for shoot tip explants was examined with three treatment combinations i.e. A - Continues sub culturing on basal medium, B - Alternate subculture on basal medium and best of establishment treatment (E₁- MS medium + 0.5 mg/l BAP + 0.1 mg/l NAA) and C - Continuous sub culturing on treatment (E₁). The results are presented in Table 4 and depicted in Plate III and Fig. 4 and 5. The culture obtained on the best establishment treatment E₁ were transferred to continuously on basal MS medium (A); alternate on basal MS medium and E₁ treatment (B) and continues on E₁ treatment (C) for four subcultures. When the shoots obtained on E₁ were transferred on the same

medium continuously for four subcultures, the multiplication rate and length of shoot declined considerably. Similar trend was seen in all the treatments. However, maximum multiplication rate and growth of shoots were reported higher in treatment B in all the subcultures as compared to other treatments. The third set of treatment (A) i.e. continuously sub-culturing the shoot to basal medium practically failed to support the multiplication of shoot. When basal medium and medium with BAP were used alternately in subsequent subcultures such no deterioration was noticed. Reuveni *et al.* (2004) used adenine sulphate in addition to BAP to improve growth and rate of multiplication.

CONCLUSION

The present investigation on “Micropropagation in papaya var. Red Lady” has clearly demonstrated its potentiality for rapid clonal propagation. It is expected that using the present protocol of micropropagation, large number of plantlets can be produced in a year starting from a single shoot tip explants. This protocol may be made commercially viable provided some work is intensified to increase the vigor and growth of the plantlets in the initial stage. The results obtained would be very much useful for mass multiplication of papaya var. Red Lady using shoot tips under local condition and proved guidelines for setting commercial unit for propagation of papaya.

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Table 1: Effect of surface sterilant treatments on establishment, contamination and growth of papaya var. Red Lady

<i>Explants: Shoot tips</i>	<i>Medium: MS</i>	<i>Incubation: 4 weeks</i>	
Treatment	Establishment Response (%)	Contamination (%)	Length of Shoot (cm)
T ₁ -HgCl ₂ (0.05 %)- 5 minutes dip	21.00(27.27)	73.00(85.70)	0.70
T ₂ -HgCl ₂ (0.05 %)-10 minutes dip	10.00(18.42)	80.00(63.45)	0.50
T ₃ -HgCl ₂ (0.1%)- 3 minutes dip	83.33(65.92)	12.00(20.26)	1.10
T ₄ -HgCl ₂ (0.1 %)- 5 minutes dip	35.00(36.27)	8.00(16.41)	0.90
T ₅ -HgCl ₂ (0.1 %)-10 minutes dip	25.00(29.98)	10.00(18.42)	0.80
T ₆ -HgCl ₂ (0.2 %)- 2 minutes dip	6.00(14.15)	10.00(18.42)	0.60
SEm ±	0.69	0.58	0.02
CD 5%	2.14	1.80	0.08
CV %	3.77	3.12	5.95

Figure in parentheses are arc sine transformed value

Table 2: Effect of surface sterilant treatments on establishment, contamination and growth of papaya var. Red Lady

<i>Explants: Axillary buds</i>	<i>Medium: MS</i>	<i>Incubation: 4 weeks</i>	
Treatment	Establishment Response (%)	Contamination (%)	Length of Shoot (cm)
T ₁ -HgCl ₂ (0.05 %)- 5 minutes dip	2.00 (8.13)	89.00 (70.83)	0.93
T ₂ -HgCl ₂ (0.05 %)-10 minutes dip	0.00 (1.28)	99.00 (84.95)	0.67
T ₃ -HgCl ₂ (0.1%)- 3 minutes dip	10.00 (18.43)	85.00 (67.55)	0.50
T ₄ -HgCl ₂ (0.1 %)- 5 minutes dip	4.73 (12.55)	69.33 (56.39)	0.80
T ₅ -HgCl ₂ (0.1 %)-10 minutes dip	12.17 (20.41)	83.33 (66.02)	1.10
T ₆ -HgCl ₂ (0.2 %)- 2 minutes dip	4.00 (11.52)	54.33 (47.49)	0.60
SEm ±	0.27	2.00	0.03
CD 5%	0.85	6.17	0.11
CV %	3.98	5.29	8.70

Figure in parentheses are arc sine transformed value

Table 3: Effect of plant growth regulators on establishment of shoot tips of papaya var. Red Lady

Medium : MS		Incubation : 4 weeks		Explants	: Shoot tips	
Treatment	Plant Growth Regulator (mg/l)			Establishment (%)	Days Taken For Establishment	Length of Shoot (cm)
	NAA	BAP	Kinetin			
E ₁	0.1	0.5	--	85.00 (67.38)	4.00	1.30
E ₂	0.1	1.0	--	67.00 (54.95)	5.67	0.90
E ₃	0.1	2.0	--	39.67 (39.03)	7.00	0.47
E ₄	0.5	0.5	--	80.00 (63.45)	6.00	0.80
E ₅	0.5	1.0	--	63.00 (52.54)	6.00	1.00
E ₆	0.5	2.0	--	39.33 (38.84)	7.00	0.55
E ₇	1.0	0.5	--	80.00 (63.44)	5.33	0.83
E ₈	1.0	1.0	--	69.67 (56.59)	7.67	0.70
E ₉	1.0	2.0	--	39.00 (38.64)	8.07	0.90
E ₁₀	0.1	--	0.5	20.00 (26.55)	5.50	1.02
E ₁₁	0.1	--	1.0	17.00 (24.33)	7.67	0.70
E ₁₂	0.1	--	2.0	0.00 (1.28)	---	0.00
E ₁₃	0.5	--	0.5	36.00 (36.87)	6.00	0.90
E ₁₄	0.5	--	1.0	30.00 (33.21)	7.00	0.80
E ₁₅	0.5	--	2.0	0.00 (1.28)	---	0.00
E ₁₆	1.0	--	0.5	13.00 (21.12)	9.83	0.30
E ₁₇	1.0	--	1.0	22.00 (27.06)	14.83	1.20
E ₁₈	1.0	--	2.0	0.00 (1.28)	---	0.00
S.Em. ±				0.79	0.18	0.02
CD 5%				2.27	0.51	0.06
CV %				3.81	5.24	5.75

Figure in parentheses are arc sine transformed value

Table 4: Effect of serial subculturing on multiplication rate of seedling shoot tip of papaya var. Red Lady

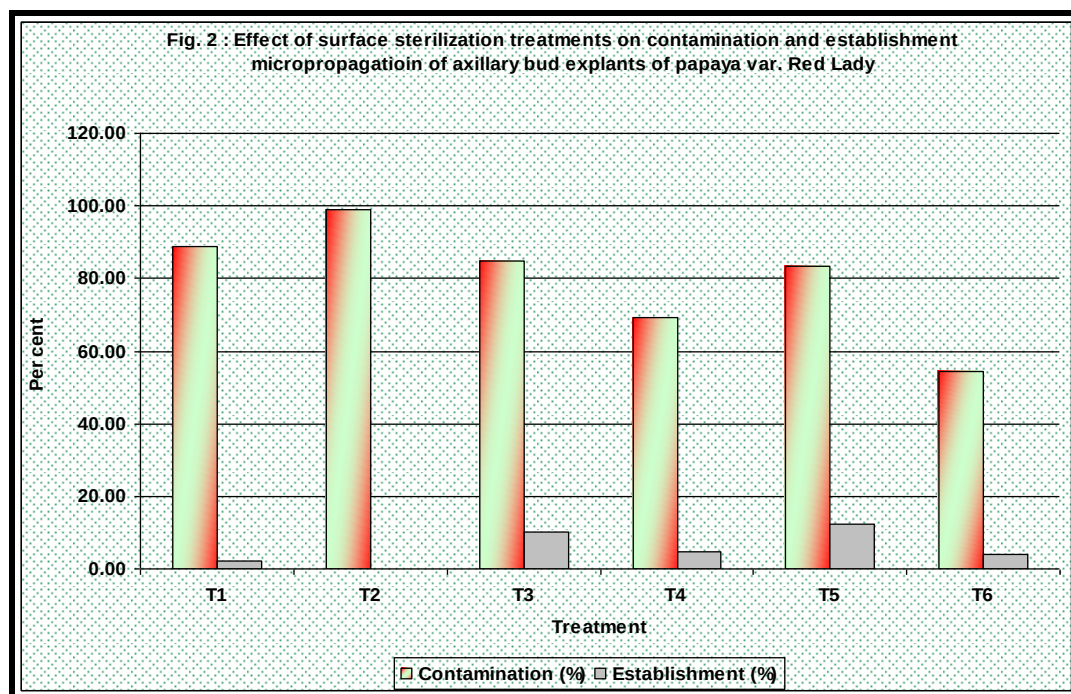
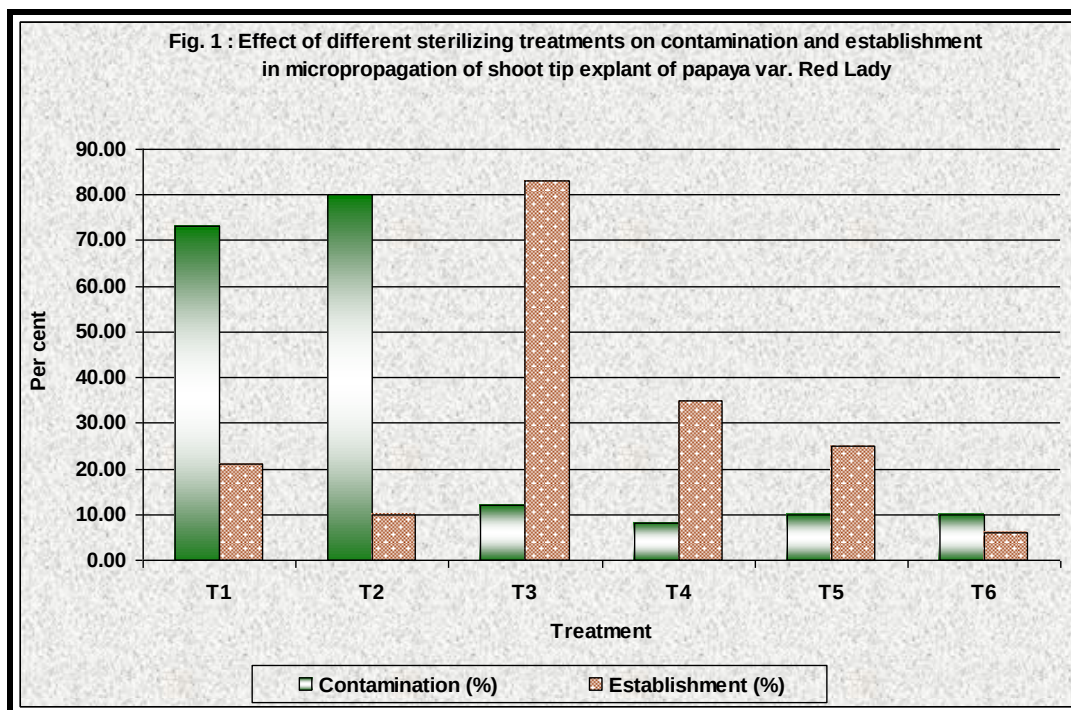
Medium : MS			Incubation : 4 weeks		Explants		: Shoot tips	
Group of Trial	1 st Sub Culturing		2 nd Sub Culturing		3 rd Sub Culturing		4 th Sub Culturing	
	Average Number of Shoot per Explants	Length of Shoot (cm)	Average Number of Shoot per Explants	Length of Shoot (cm)	Average Number of Shoot per Explants	Length of Shoot (cm)	Average Number of Shoot per Explants	Length of Shoot (cm)
A	3.00	1.30	1.49	1.00	1.00	1.20	1.01	0.81
B	4.50	1.40	4.00	1.30	3.50	1.30	3.41	1.17
C	5.00	1.27	3.61	1.00	2.60	1.00	2.51	1.10
S.Em.±	0.08	0.03	0.04	0.02	0.05	0.02	0.04	0.01
CD 5%	0.24	0.09	0.12	0.06	0.15	0.75	0.12	0.55
CV %	5.31	6.52	3.62	5.25	5.81	5.71	4.48	4.74

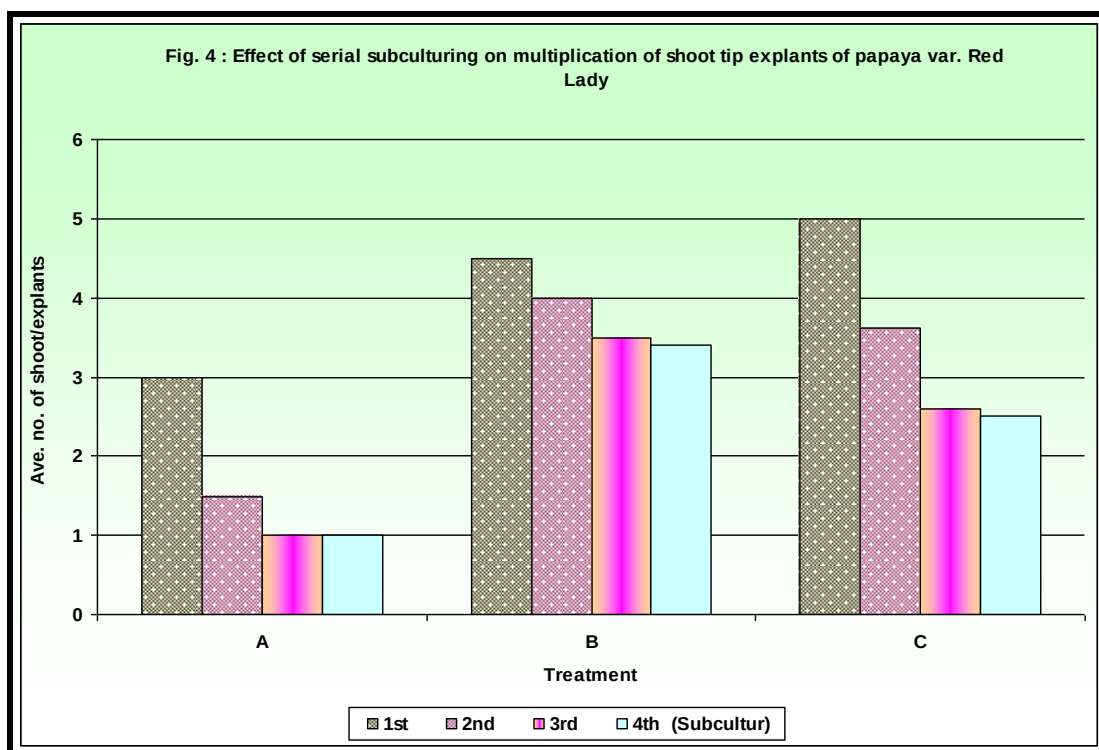
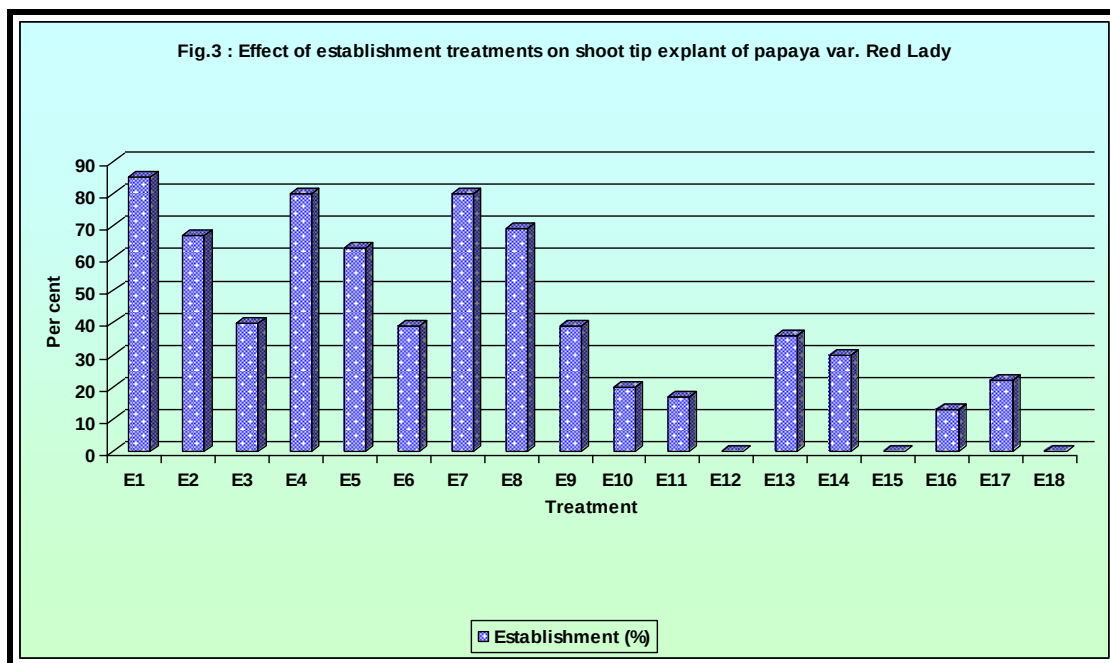
A- Continues sub culturing on basal medium.

B- Alternate subculture on basal medium and best of establishment treatment (E1)

C - Continuous sub culturing on treatment (E1).

E1- MS medium + 0.5 mg/l BAP + 0.1 mg/l NAA.





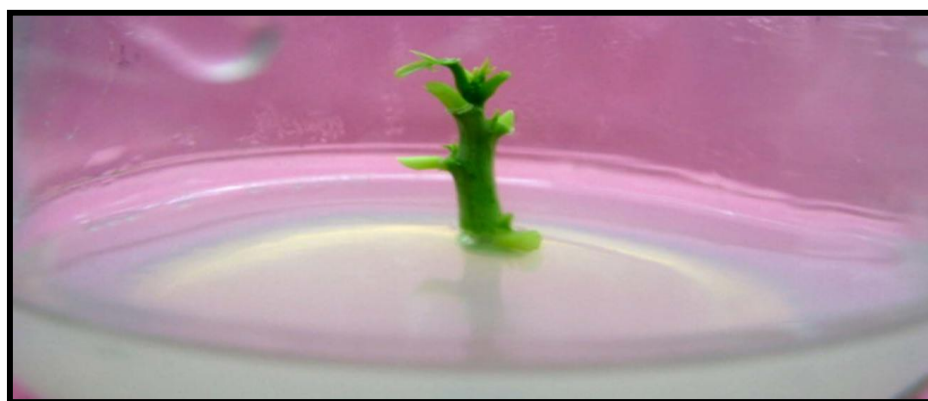
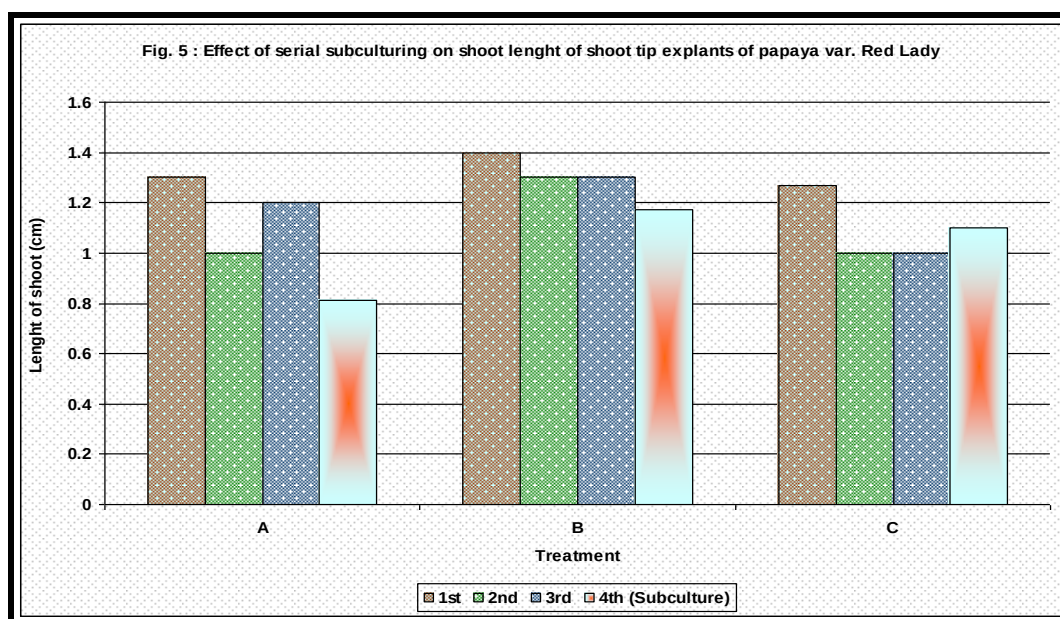


Plate 1. Establishment of shoot tip explants with sterilant treatment T_3 - $HgCl_2$ 0.1% for 3 min dip



Plate 2. *In vitro* shoot multiplication from shoot tip



Plate3. Serial subculturing of shoots *in vitro*

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