INTERNATIONAL JOURNAL OF PLANT PROTECTION | VOLUME 6 | ISSUE 2 | OCTOBER, 2013 | 449-456

IJPP

RESEARCH ARTICLE

In vitro micro propagation of papaya var. Red Lady

■ J.R. PATEL, R.M. PATEL, S.R. PATEL AND VIRENDRA SINGH*

College of Agriculture, Navsari Agricultural University, BHARUCH (GUJARAT) INDIA

ARITCLE INFO

Received : 06.08.2013
Revised : 08.10.2013
Accepted : 20.10.2013

Key **W**ords :
Explants, *In vitro*, Shoot tip sterilization, Papaya rooting

ABSTRACT

Papaya is propagated through seed. It is a cross-pollinated crop, the plant raised from seeds have a mixed inheritance which make them highly variable in performance. Clonal propagation is an urgent necessity for improvement of papaya. Although, desirable characteristics of papaya var. Red Lady, growers are not able to adopt this variety due to very high cost of seed. 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, pH of the medium, sucrose and adenine sulphate concentration in the medium. In multiplication study, the maximum shoot multiplication was observed in alternate sub culturing in basal medium and MS medium + 0.5 mg/l BAP + 0.1 mg/l NAA and gave highest number of shoots per explants. Sucrose 30 g/l in medium was found to be more favourable for maximum number of shoot and length of longest shoots. Out of various pH level tested, pH 5.7 recorded maximum numbers of shoots (3) and maximum length of longest shoots (2.75 cm). In proliferation medium, maximum length of shoot, numbers of shoots and growth rate was observed in MS medium fortified with 160 mg/l adenine sulphate. *In vitro* rooting occurred on shoot regeneration medium; however, it was a slow process. Rooting treatment consisting of half MS medium supplemented with 1.0 mg/l IBA was found to be the best for early induction of roots (28 days), maximum number of roots/shoot (5.00) and length of root (6.00 cm) also. Among all potting mixtures tested, the soil: sand: FYM (1/1/1: V/V/V) was found to be suitable for hardening *in vitro* raised papaya plantlets.

How to view point the article : Patel, J.R., Patel, R.M., Patel, S.R. and Singh, Virendra (2013). *In vitro* micro propagation of papaya var. Red Lady. *Internat. J. Plant Protec.*, **6**(2) : 449-456.

***Corresponding author:**
Email: singh_nau@yahoo.com

INTRODUCTION

There is tremendous scope of developing fruit industry in India. The papaya (*Carica papaya* L.) belongs to family Caricaceae. The edible fruit are available with *Carica* genus (Muthukrishnan and Irulappan, 1990). Papaya is a native of tropical of North and South America (Litz,1984). Normally, papaya (Red Lady) is propagated through seed. No male plant hence all produced fruits. Weight about 1.5-2.0 kg. Flesh is thick red with 13% sugars content, delicious taste and excellent aroma. It is a cross-pollinated crop, the plant raised from seeds have a mixed inheritance which make them highly variable in performance. Although, desirable characteristics of var. Red Lady, the growers are not able to adopt this variety due to very high cost of seed. The importance of these problems is evident the lack of true to-type cultivars at present. Clonal propagation is an urgent necessity for improvement of papaya. Similarly, in spite of careful realization of treatments against pest and diseases, bacterial and virus infections cannot be prevented totally. The answer of these problems is expected through plant tissue culture techniques (Micro propagation). Papaya is one of the few fruiting plants of commercial value to be propagated *in vitro* tissue culture. Various attempts have been made to propagate papaya *in vitro* though callus regeneration, somatic embryogenesis (Litz and Conover, l982) and shoot proliferation (Rajeevan and Pandey, l983). Research work on different aspects of papaya tissue culture has been reviewed by Litz (1984).

MATERIAL AND METHODS

Present investigation on micro propagation in papaya var. Red Lady was carried out at Department of Biotechnology, Tissue Culture Laboratory, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari. While standardizing the methods for micropropagation in papaya, different factors were studied *i.e.* sources of explants (shoot tip and axillary bud), surface sterilant ($HgCl_2$: 0.05% each for 5 and 10 min; 0.1% each for 3 and 5 min and 0.2 % for 2 min dip). Shoot tip explants were sterilized with sterilants and 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 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/1 sucrose and 8 g/1 agar for all factors. The cultures were incubated at 26 ± 2^{o}C in culture room. The culture vessels and media were autoclaved at pressure of 15 lb/ inch^2 for 20 minutes at approximately 121^{o}C temperature. After which autoclaved media were transferred to an air–conditioned room for storage before use.

Standardization of proliferation medium :

Different plant growth regulators were tried to standardize the most suitable culture proliferation medium for papaya var. Red Lady. Total 21 treatment combinations were studied as are under:

W: Best NAA treatment for establishment

Light intensity: L_1-1000 Lux, L_2-2000 Lux, L_3-3000 Lux

P_1 to P_3 = MS + W + L_1, L_2, L_3

P_4 to P_6 = MS + W + BAP 0.5 mg/l + L_1, L_2, L_3

P_7 to P_9 = MS + W + BAP 1.0 mg/l + L_1, L_2, L_3

P_{10} to P_{12}= MS + W + BAP 2.0 mg/l + L_1, L_2, L_3

P_{13} to P_{15}= MS + W + KN 0.5 mg/l + L_1, L_2, L_3

P_{16} to P_{18}= MS + W + KN 1.0 mg/l + L_1, L_2, L_3

P_{19} to P_{21}= MS + W + KN 2.0 mg/l + L_1, L_2, L_3

Standardization of optimum sucrose for shoot growth:

Experiment was conducted to study the effect of different levels of sucrose *i.e.* S_1 to S_5 (1.0, 2.0, 3.0, 4.0 and 5.0%) on proliferation rate of shoot. MS medium supplemented with 1.0 mg/l kinetin +0.1 mg/l NAA at 3000 Lux light intensity and solidified with 0.8 per cent agar was used.

Standardization of optimum pH for *in vitro* shoot growth:

Experiment was framed to study the effect of different pH of the medium *i.e.* I_1 to I_6 (4.5, 5.0, 5.5, 5.7, 6.0 and 6.5) on proliferation rate. MS medium supplemented with 1.0 mg/l kinetin 0.1 mg/l + NAA at 3000 Lux light intensity and solidified with 0.8 per cent agar was used. The pH was adjusted with 0.1N HCl or 0.1N NaOH as found necessary before autoclaving.

Standardization of optimum adenine sulphate in medium:

A trial was conducted to study the effect of different levels of adenine sulphate *i.e.* A_1 to A_4 (40, 80, 120 and 160 mg/ l)for maximum shoot growth. MS medium supplemented with 1.0 mg/l kinetin +0.1 mg/l NAA at 3000 Lux light and solidified with 0.8 per cent agar was used.

Standardization of *in vitro* rooting medium:

The trial on *in vitro* rooting was conducted on quadric, half and full MS medium gelled with 0.8 per cent agar. Each medium was supplemented with different concentration of IBA. Total 9 treatments combinations were studied are as follow:

R_1 to R_3 = MS ¼ + 0.5, 1.0 and 2.0 mg/l IBA

R_4 to R_6 = MS ½ + 0.5, 1.0 and 2.0 mg/l IBA

R_7 to R_9 = MS + 0.5, 1.0 and 2.0 mg/l IBA

Standardization of potting mixture for hardening of *in vitro* plants :

Rooted plantlets were taken out from the culture vessels with the help of forceps. The rooted plantlets were then, dipped in 0.05 per cent bavistin, (carbendazim 50 per cent WP) and planted in earthen pots containing pretreated cocopeat. They were covered with glass beaker continuously for 6-7 days and kept in air conditioned room. Subsequently, plantlets were kept for 15 days in shadow condition for proper hardening to natural environment. For hardening, total five treatments were tested *i.e.* H_1 to H_5 (Vermiculite, Cocopeat, FYM: soil: sand (1/ 1/1:V/V/V), sand and perlite.

RESULTS AND DISCUSSION

Standardization of proliferation medium:

The data on proliferation response to different level of BAP, Kinetin and NAA supplemented in MS medium in combination of different light intensity are presented in Table 1, Fig. 1 and Plate-1. It was noticed that maximum proliferation (67 %) was recorded in treatment MS medium + 1.0 mg/l kinetin + 0.1 mg/l NAA at 3000 Lux light intensity (P_{18}) followed by P_6 and P_{17}, which was 62.00 and 59.00 per cent, respectively. Maximum length of internodes was recorded in P_{18} (0.60 cm) followed by treatment P_9 (0.55 cm). Similarly, length of shoot was significantly higher in treatment P_{18} (2.20 cm) followed by treatment P_9 (1.90 cm). Higher level of cytokinins to auxin favoured shoot bud induction where as, an opposite condition *i.e.* an increased concentration of auxin and low cytokinins promoted roots (Skoog and Miller, 1957). In most of the studies of *in vitro* culture of papaya, basal media derived from the MS (Beniwal *et al.*, 2006; Reuveni *et al*., 2004; Suthamathi *et al.*, 2002; Rahaman *et al.*, 1992; Dinesh Babu *et al*., 2000). Light intensity exhibited more effective role on the proliferation of shoot in papaya by earlier workers (Suthamathi *et al.*, 2002;

Plate 1 : MS medium supplemented with 1.0 mg/l Kinetin + 0.1 mg/l NAA at 3000 Lux light intensity

Reuveni *et al*., 2004).

Effect of level of sucrose in medium on shoot proliferation:

From the perusal of data given in Table 2, it is evident that the proliferation was directly affected by the different concentrations of sucrose in the medium. Maximum number of shoots per explant and length of longest shoot was observed in 3 per cent sucrose level (S_3) that is 2.2 and 2.0 cm, respectively (Fig. 2, Plate 2). In general, maximum proliferation was seen at S_3 treatment; moreover, it was reduced gradually at either increased or decreased levels of sucrose from S_3. The culture growth is influenced by the source of carbon energy, which is inheritable in any culture medium. Kumar and Kumar (1998) reported that level of sucrose was maintained between 2 to 3 per cent in majority of medium. Further, in papaya glucose 1.5 per cent + fructose 1.5 per cent in medium gave the best response to

Table 1 : Effect of BAP, KN, and NAA with different light intensity on shoot proliferation of papapa var. Red Lady

Treatment no.	Plant growth regulator			Light intensity (Lux)	Proliferation (%)	Length of internodes per shoot (cm)	Length of shoot (cm)
	NAA (mg/l)	BAP (mg/l)	KN (mg/l)				
P_1	0.1	--	--	1000	0.00 (1.28)	0.00	0.00
P_2	0.1	--	--	2000	0.00 (1.28)	0.00	0.00
P_3	0.1	--	--	3000	0.00 (1.28)	0.00	0.00
P_4	0.1	0.5	--	1000	40.83 (39.72)	0.25	1.20
P_5	0.1	0.5	--	2000	44.67 (41.94)	0.45	1.60
P_6	0.1	0.5	--	3000	62.00 (51.96)	0.51	1.70
P_7	0.1	1.0	--	1000	46.00 (42.70)	0.41	1.20
P_8	0.1	1.0	--	2000	45.00 (42.13)	0.54	1.50
P_9	0.1	1.0	--	3000	49.00 (44.43)	0.55	1.90
P_{10}	0.1	2.0	--	1000	30.00 (33.21)	0.33	1.20
P_{11}	0.1	2.0	--	2000	57.00 (49.03)	0.34	0.80
P_{12}	0.1	2.0	--	3000	52.00 (46.15)	0.38	1.80
P_{13}	0.1	--	0.5	1000	36.00 (36.87)	0.30	0.70
P_{14}	0.1	--	0.5	2000	38.00 (38.05)	0.39	1.20
P_{15}	0.1	--	0.5	3000	44.00 (41.55)	0.48	0.90
P_{16}	0.1	--	1.0	1000	38.00 (38.05)	0.35	1.40
P_{17}	0.1	--	1.0	2000	59.00 (50.19)	0.55	1.80
P_{18}	0.1	--	1.0	3000	67.00 (54.95)	0.60	2.20
P_{19}	0.1	--	2.0	1000	45.00 (42.13)	0.20	0.90
P_{20}	0.1	--	2.0	2000	52.00 (46.15)	0.34	1.00
P_{21}	0.1	--	2.0	3000	59.00 (50.19)	0.32	1.20
SEm ±					0.68	0.01	0.03
CD (P=0.05)					1.94	0.32	0.11
CV %					3.12	5.60	5.48

Figure in parentheses are arc sine transformed value
Medium : MS, Incubation : 4 weeks, Explants : Shoots tips

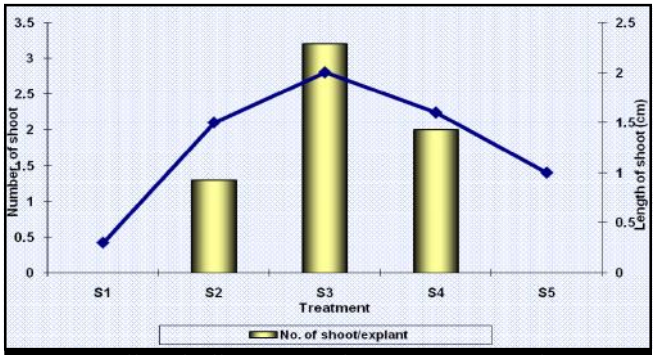

Fig. 2 : Effect of different levels of sucrose on number of shoots and length of shoot in proliferation medium of papaya var. Red Lady

Plate 2: Proliferation on shoots on 3% sucrose in the medium

growth of shoot (Naik, 1997).

Effect of pH on shoot proliferation:

The influences of pH on proliferation rate, data are presented in Table 3. Different pH levels tested were 4.5, 5.0, 5.5, 5.7, 6.0 and 6.5. Out of all pH levels tested, maximum number of shoots per explant (3.0) and length of longest shoot (2.75 cm) were recorded with pH 5.7 among all the treatments. The trends of number of shoots per explant and length of longest shoot increased as pH level increased up to 5.7, then, subsequently declined (Fig. 3, Plate 3). Tissue culture of majority of fruit crops are grown satisfactory at pH 5.6 to 5.8 (Conger, 1987). In the present investigation, maximum proliferation rate and best growth were observed at pH 5.7, and decreased at lower as well as higher pH value. Effect of pH on proliferation rate and growth of culture is supported by many workers such as anthurium (Sunila

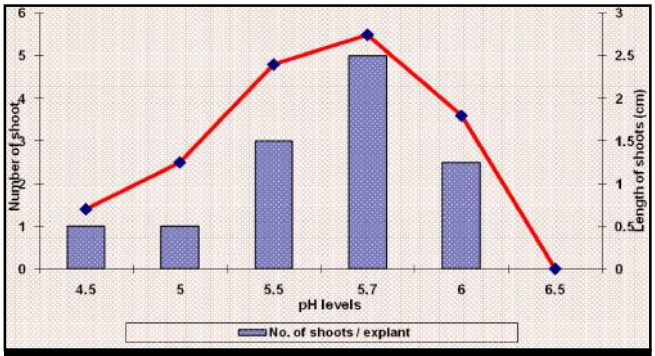

Fig. 3 : Effect of different pH levels on number of shoots and length of shoot in proliferation medium of papaya var. Red Lady

Table 2: Effect of different level of sucrose on *in vitro* shoot proliferation of papaya var. Red Lady

Treatment No.	No. of shoots/explant	Length of longest shoot (cm)
S_1 - Sucrose (1 %)	0	0.3
S_2 - Sucrose (2 %)	1.3	1.5
S_3 - Sucrose (3 %)	2.2	2.0
S_4 - Sucrose (4 %)	2	1.6
S_5 - Sucrose (5 %)	0	1

Medium- MS + 1.0 mg/l kinetin + 0.1 mg/l NAA at 3000 Lux light intensity
Medium : MS, Incubation : 4 weeks, Explants : Shoots tips

Table 3 : Effect of pH level of the medium on shoot proliferation of papaya var. Red Lady

Treatment No.	pH level	No. of shoots / explant	Length of longest shoots (cm)
I_1	4.5	1	0.7
I_2	5.0	1	1.25
I_3	5.5	3	2.4
I_4	5.7	3	2.75
I_5	6.0	2.5	1.8
I_6	6.5	0	0

Medium- MS + 1.0 mg/l Kinetin + 0.1 mg/l NAA at 3000 Lux light intensity
Medium : MS, Incubation : 4 weeks, Explants : Shoots tips

Plate 3: Proliferation on shoots on the medium adjusted at 5.7 pH

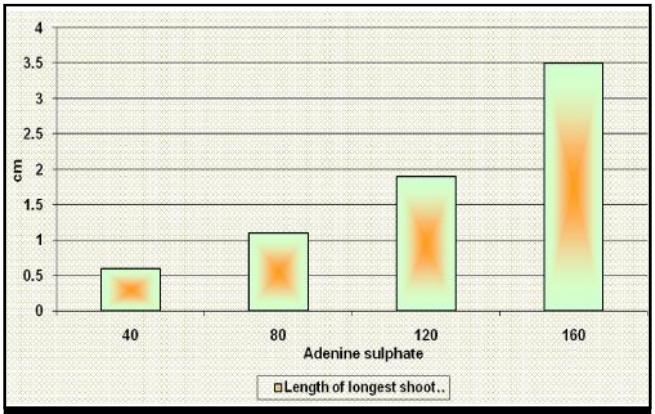

Fig. 4 : Effect of adenine sulphate in proliferation medium on shoot length and growth of papaya var. Rad Lady

Kumari, 2006); citrus (Desai, 1994); papaya (Naik, 1997) and tuberose (Thosar, 1997).

Effect of adenine sulphate of the medium on shoot proliferation:

It is evident from the Table 4, that proliferation and growth of shoots were influenced by adenine sulphate. The longest shoot length (3.5 cm) was obtained at high concentration (160 mg/l) adenine sulphate in medium while lowest level (40 mg/l) of adenine sulphate registered minimum shoot length (0.6 cm). (Fig. 4, Plate 4). This finding is in accordance with those of Reuveni *et al*. (2004).

Effect of IBA and strength of the medium on *in vitro* of rooting in shoot:

The data on rooting response to different levels of IBA supplemented in quarter, half and full strength of MS medium is presented in Table 5. It was noticed that rooting of *in vitro* shoot on half strength MS medium was better in respect to all the rooting characters than that observed on full and quarter strength MS medium. The best rooting was observed in

Table 4: Effect of adenine sulphate of medium on shoot proliferation of papaya var. Red Lady

Treatment No.	Adenine sulphate (mg/l)	Growth of culture	Length of longest shoots (cm)
A_1	40	+	0.6
A_2	80	++	1.1
A_3	120	+++	1.9
A_4	160	++++	3.5

Medium- MS + 1.0 mg/l Kinetin + 0.1 mg/l NAA at 3000 Lux light intensity
+ = Poor growth ++ = Good growth +++= Very growth ++++ = Excellent growth
Medium : MS, Incubation : 4 weeks, Explants : Shoots tips

Table 5 : Effect of IBA and strength of medium on induction of rooting of papaya var. Red Lady

Treatment No.	Rooting (%)	Days taken for root initiation	Length of root (cm)	No. of root / shoot	Length of shoot (cm)
R_1	0.00 (1.28)	–	0.00	0.00	0.00
R_2	65.00 (53.73)	31.67	2.00	2.00	2.50
R_3	69.00 (56.17)	36.00	0.50	2.00	1.97
R_4	0.00 (1.28)	–	0.00	0.00	0.00
R_5	78.00 (62.03)	28.00	6.00	5.00	4.50
R_6	70.00 (56.79)	32.67	2.00	4.00	0.97
R_7	0.00 (1.28)	–	0.00	0.00	0.00
R_8	60.00 (50.77)	35.33	4.50	3.00	3.23
R_9	50.33 (45.19)	37.33	3.00	2.00	2.23
S.Em. ±	0.57	1.63	0.05	0.05	0.04
CD (P=0.05)	1.70	4.86	0.16	0.16	0.13
CV %	2.73	12.69	4.71	4.71	4.50

Figure in parentheses are arc sine transformed value
Incubation : 6 weeks

Plate 4: Development of shoot in the medium containing adenine sulphate 160 mg/l

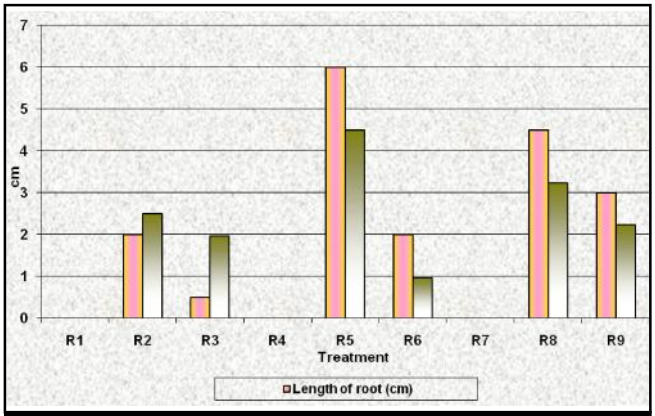

Fig. 5 : Effect of strength of medium and IBA concentrations on rooting in shoot of papaya var. Red Lady

treatment (R_5) half MS medium containing 1.0 mg/l IBA (Fig. 5, Plate 5). Moreover, no response of rooting was reported at lowest level of IBA (0.5 mg/l) in the all strength of MS mediums. The response of rooting was increased as increased the IBA levels in all the strength of MS medium. Minimum days taken for root initiation was found in treatment R_5 (28 days) followed by R_2 (31.67 days). Similarly, length of root was highest in treatment R_5 (6.00 cm) followed by R_8 (4.5 cm). Maximum number of root/shoot was also observed in R_5 followed by R_6 treatment. The response of rooting was decrease with either increased or decreased the level of IBA. Bannok *et al*. (1989) in pear and Yadav *et al*. (1990) in black plum observed reduction in rooting response at higher concentration of auxin. The present finding is supported by those of Burikam *et al*. (1988), Rahaman *et al*. (1992), Mondal *et al*. (1994), Dinesh Babu *et al*. (2000), Suthamathi *et al*. (2002), Beniwal *et al*. (2006), in papaya.

Plate 5: Best rooting observed in half strength MS medium containing 1.0 mg/l IBA

Effect of different potting mixtures on survival of *in vitro* raised plantlets:

The survival rate of plantlet was significantly influenced by potting mixture (Table 6, Fig. 6 and Plate 6). The maximum survival per cent of plantlet (70 %) was observed in treatment H_3 (FYM: soil: sand, V/V/V) followed by H_1 (Vermiculite) and H_2 (Coco peat). Significantly minimum days taken for

Table 6: Effect of different potting mixtures on hardening of papaya var. Red Lady

Treatment No.	Potting mixture	Survival of Plantlets (%)	Days taken for establishment	Length of shoot (cm)
H_1	Vermiculite	63.00 (52.44)	9.75	7.60
H_2	Cocopeat	49.00 (44.43)	13.00	7.00
H_3	FYM: Soil: Sand (1:1:1 :: V/V/V)	70.00 (56.80)	8.00	8.30
H_4	Sand	20.00 (26.54)	16.00	6.80
H_5	Perlite	45.00 (42.14)	11.00	6.50
S.Em. ±		0.84	1.01	0.14
CD (P=0.05)		2.55	0.33	0.45
CV %		3.82	5.81	4.13

Figure in parentheses are arc sine transformed value.

establishment sprouting and was observed in H_3 (8.00 days) treatment which was followed by H_1 (9.75 days). Similarly maximum length of shoot was registered in treatment H_3 (8.30 cm) followed by H_1 (7.60 cm) and H_2 (7.00 cm). *In vitro* plantlets are delicate as they are raised in controlled conditions require being hardened before transplanted in the field. These observations are supported by various earlier workers (Rahaman *et al.*, 1992; Dinesh Babu *et al.*, 2000; Suthamathi *et al.*, 2002) The hardened plants were successfully transplanted in the field where they are growing equally or better than conventionally propagated material (visual observation).

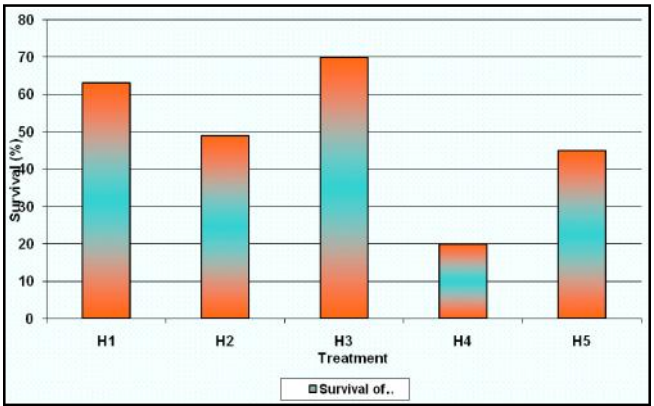

Fig. 6 : Effect of different potting mixtures on survival of plantlets of papaya var. Red Lady

Plate 6: The maximum survival and highest length of shoot observed in treatment FYM + Soil + Sand)

Conclusion :

It estimated that using the present protocol of micropropagation, large number of plantlets can be produced in a year starting from a single shoot tip explant. 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 after transplanting and testing the plantlets in field conditions, besides some scaling up techniques for large scale production. 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.

REFERENCES

Bannok, Mayashi, S. and Tanabe, K.(1989). *In vitro* propagation of Japanese pear cultivars. *J. Jap. Soc. Hort. Sci*., **58** (1) : 37-42.

Beniwal, V. S, Sehrawat, S. K., Dahiya, D. S., Benival, L. S. and Singh, S. (2006). Effect of season on *in vitro* regeneration of papaya. *Haryana J. Hort. Sci*., **53** (1 & 2): 35-37.

Burikam, S., Chommalee, V. and Attathom, S. (1988). Effect of plant growth regulators on papayas (*Carica papaya*) cultured *in vitro*. *Kesetsart J.(Nat. Sci. Suppl.),* **22** (5) : 1-6.

Conger, B.V. (1987). *Cloning agriculture plants viz., in vitro* techniques CRC Press. Florida.

Desai, R.A. (1994). *In vitro* propagation studies on citrus-acid lime var. Kagzi Lime. M.Sc. (Hort.) Thesis, Gujarat Agricultural University, S.K. Nagar, GUJARAT (INDIA).

Dinesh Babu, Sathiamoorthy and Chezhiyan (2002). Effect of age mother trees on *in vitro* establishment of axillary buds of papaya. (*Carica papaya* L.). *South Indian J. Hort*., **48**(1-6): 23-25.

Litz, R. E. (1984). Papaya. In: *Handbook of plant cell culture* (Sharp, W. R., Evans, D. A., Ammirato, P. V. and Yamada, Y. Eds.) Macmillon Publishing Co., New York, pp. 349-368.

Litz, R.E. and Conover, R.A. (1982). *In vitro* somatic embryogenesis and plant regeneration from *Carica papaya* L. ovular callus. *Plant Sci. Letters*, **26** (2-3) : 153-158.

Mondal, M., Gupta, S. and Mukherjee, B.B. (1994). Callus culture and plantlet production in *Carica papaya* L. (Var. Honey Dew). *Plant Cell Rep*., **13** (7) : 390-393.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant*, **15** (3) : 473-497.

Muthukrishna, C. R. and Irrulappan I. (1990). Papaya. In: *Fruits: Tropical and subtropical* (Bose, T.K. and Mitra, S.K. Eds), Naya Prakash Calcutta, pp. 304-355.

Naik, U.I. (1997). Micropropagation of papaya (*Carica papaya* L.) var. Honey Dew. M.Sc. (Ag.) Thesis, Gujarat Agricultural University, S.K. Nagar, GUJARAT (INDIA).

Rahaman, S. M., Hossain, M., Joarden, O. I. and Islam, R. (1992). Rapid clonal propagation of papaya through cultivar of shoot apices. *Indian J. Hort*., **49**(1): 18-22.

Reuveni, O., Shlesinger, D.R. and Lavi, U. (2004). *In vitro* clonal propagation of dioecious *Carica papaya. Pl. Cell Tissues Organ Cult*., **20**(1): 41-46.

Skoog, F. and Miller, C.O. (1957). Chemical regulation of growth and organ formation in plant tissue cultured *in vitro*. *Symp. Sec. Exp. Biol*., **11**: 118-130.

Sunila Kumari, (2006). *In vitro* studies in *Anthurium andreanum* M.Sc. (Hort.) Thesis the Navsari Agricultural University, Navsari, GUJARAT (INDIA).

Suthamathi, S.J., Haripya, K. and Kamalakannan, S. (2002). Micropropagation cv. CO-5. *Indian J. Hort*., **59**(1): 13-16.

Thosar, A.P. (1997). Micropropagation of tuberose (*Polianthes tuberose* L.) var. Local Double. M.Sc. (Ag.) Thesis, Gujarat Agricultural University, S.K. Nagar, GUJARAT (INDIA).

Yadav, U., Lal, M. and Jaiswal, V.S. (1990). *In vitro* micropropagation of the tropical fruit tree. *Syzigium cumini* L. *Plant Cell Tissue & Organ Culture*, **21** (1) : 87-92.