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Studies on the Effect of Growth Regulators on *In-vitro* cloning of *Carica papaya* L. through Shoot Tip and Inflorescences

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A B S T R A C T

Papaya is the most imp**o**rtant fruit crop of tropical and sub-tropical countries of the world. It is valued for its nutritional as well as pharmaceutical importance as it is a rich source of provitamin A, Calcium and Papaina photolytic enzyme. Since Papaya is highly heterozygous fruit crop. There is a need for multiplication of plant through Tissue Culture for bearing fruits in almost all the plants. Keeping these facts in view the multiplication of Papaya *Carica papaya* L. cv. Pusa Delicious through Tissue Culture is needed for the production of numerous plants within a short period that is why the present experiment was carried out in the Tissue Culture Lab of the Department of Horticulture, Sardar Vallabhbhai University of Agriculture & Technology, Meerut, (U.P.) during the year 2016-2017. The experiment was conducted in two way factorial design with 33 treatments comprising of 3 level of BAP (1,2 and 3 ppm), 3 levels of Kinetin(1, 1.5 and 2 ppm), 6 level of NAA(0.01, 0.1, 0.5, 1, 2 and 3 ppm) and 5 levels of IBA(0.1, 0.5, 1, 2 and 3 ppm) replicated thrice. The different concentrations of Auxins and Cytokinins alone and in various combinations are applied. The effect of these combinations was observed among the different concentration of the bio-regulators, BAP 1 ppm + NAA 0.5 ppm was found to be the best for shoot development and as well as for maximum number of shoot. In case of rooting of explants, the effect of IBA and NAA was studied with the half dose of CHU (N6) media, NAA 3 ppm was most effective in terms of root induction in explant which induced the root just in 11.66 days. IBA 1 ppm was best in inducing maximum number of roots. Hence based on result obtained from the investigation it can be concluded that the combination of BAP 1 ppm and NAA 0.5 ppm is the best for shoot development and NAA 3 ppm and IBA 1 ppm is the best for early rooting and maximum rooting respectively in CHU (N6) media for Papaya (*Carica papaya* L.) tissue culture.

Introduction

Papaya (*Carica papaya* L.), the fifth important fruit of India, is valued for the nutritional qualities of its fruit as a source of provitamin A and calcium as well as for the pharmaceutical industry as a rich source of commercial papain. Almost all the plant parts including leaves, fruits, seeds, latex, bark and roots are used in several ways (Anibijuwon and Udeze 2009). The usefulness of papaya extends beyond as a raw material in the food

and pharmaceutical industry; its potential applications and uses are yet to be fully explored, derived and understood (Boshra and Tajul, 2013).

A big disadvantage regarding its commercial cultivation is its dioecious character and heterozygosity coupled with cross pollination. Thus, the conventional method of its propagation through seeds results in great variability in fruit quality. Since colour and flavour of its fruit are controlled by pollen grains, cloning through tissue culture of female and male elite plants is warranted to ensure uniform quality and yield of fruit. There are several reports on tissue culture multiplication of this plant, including genetic transformation. It has been micro propagated mostly by using seedling explants, but also through shoot tips taken from mature plants. There are quite a few reports on efficient micro propagation of papaya, they are in alien varieties. As expected, such protocols are not equally applicable in case of indigenous varieties in view of the wide variation existing in the *in-vitro* responses of different varieties and clones in general. The poor transplant success of the *in-vitro* raised plantlets, which in turn depends on difficulty in obtaining desirable rooting of micro shoots is a big hindrance in its commercial propagation. Furthermore, establishment of aseptic cultures through shoot apices of mature plants is intractable because of high incidence of endogenous infection. This paper reports cloning of mature plants of *Carica papaya* L. var. Pusa Delicious of known sexuality employing tips of their shoots and young inflorescences. There appears to be no earlier report on the latter aspect.

The western Uttar Pradesh may be well suited for disease free commercial cultivation of papaya. Since papaya is highly heterozygous fruit crop. There is need of multiplication of plants through tissue culture for bearing fruits in almost all the plants. Viewing the above fact the multiplication of papaya through tissue culture is needed for the production of numerous plants within a short period of time that is why the present experiment is proposed.

Materials and Methods

Plant materials and in vitro culture

The healthy shoot tips of 1 to 1.5-month-old papaya seedlings and immature female flower were taken from papaya experimental block of Horticultural Research Centre (HRC), Sardar Vallabhbhai Patel University of Agriculture and Technology Meerut (U.P).Cloning of female papaya plants through *in-vitro* shoot bud culture is an ideal approach (Ali 1976).Plants were grown from fresh seed obtained from mature fruit. Apical shoot tips 2cm in length were removed and the leaves were excised leaving the petioles intact (Arora and Singh, 1978). The excised shoot tips were then treated for 15 min in a 1% sodium hypochlorite solution containing a few drops of 7X detergent. After three rinses in sterile water, apical bud explants of 0.25-0.5cm were excised and placed on nutrient media. The basal medium for papaya was medium concentrations of minerals and growth factors, riboflavin and containing 2% sucrose and 0.8% Agar. The pH of all media was adjusted to 5.8 with 0.1 M KOH before autoclaving at 121°C for 15 min. Shoots were cultured in polycarbonate screw cap containers and incubated at 25 ° ± 1°C, with cool white fluorescent tubes providing a photoperiod of 16 h day and 8 h darkness.

Length of shoot at different time interval

It is evident from the data presented in Table 1 that the length of the shoot was significantly affected by the various concentrations of growth hormones supplemented in the CHU

(N6) media, the data was recorded after 4 weeks, 6 weeks, 8 weeks and 10 weeks after the shoot tip and inflorescence inoculated in the media. Maximum length of shoot 4 weeks after inoculation was recorded with application of BAP 1ppm + NAA 0.5ppm (1.5 cm) followed by Kinetin 2ppm + NAA 0.01ppm (1.3 cm). While minimum length of shoot was recorded BAP 3ppm + Kinetin 2ppm (0.2 cm).

Table.1 Number of shoot per explant, Length of shoot

Treatment	Treatment (ppm)	Number of Shoot/explant	Length of shoot (cm)			
			4 Weeks	6 Weeks	8 Weeks	10 Weeks
T_1	BAP 1 + Kinetin 0	13.66	0.4	0.6	1.0	1.9
T_2	BAP 2 + Kinetin 0	11.66	0.6	0.9	1.7	2.6
T_3	BAP 3 + Kinetin 0	8.33	0.5	0.8	1.6	2.5
T_4	BAP 1 + Kinetin 1	18.33	0.7	1.0	1.8	2.9
T_5	BAP 2 + Kinetin 1	16.66	0.8	1.1	1.9	2.8
T_6	BAP 3 + Kinetin 1	14.00	0.6	0.9	1.7	2.75
T_7	BAP 1 + Kinetin 1.5	15.33	1.0	1.3	2.1	2.95
T_8	BAP 2 + Kinetin 1.5	9.33	0.9	1.2	2.0	2.99
T_9	BAP 3 + Kinetin 1.5	7.00	0.8	1.1	1.9	2.89
T_{10}	BAP 1 + Kinetin 2	12.33	0.8	1.0	1.8	2.79
T_{11}	BAP 2 + Kinetin 2	10.00	1.1	1.4	2.2	2.9
T_{12}	BAP 3 + Kinetin 2	6.66	0.2	0.5	0.85	1.56
T_{13}	BAP 0 + Kinetin 1	11.66	0.7	1.1	1.9	2.94
T_{14}	BAP 0 + Kinetin 1.5	12.33	0.8	1.2	2.0	2.10
T_{15}	BAP 0 + Kinetin 2	9.00	0.9	1.5	2.1	2.8
T_{16}	BAP 1 + NAA 0.01	15.66	0.4	0.8	1.6	2.9
T_{17}	BAP 2 + NAA 0.01	14.33	0.3	0.7	1.5	3.0
T_{18}	BAP 3 + NAA 0.01	11.00	0.2	0.7	1.4	2.8
T_{19}	BAP 1 + NAA 0.1	16.66	0.5	0.9	1.7	3.3
T_{20}	BAP 2 + NAA 0.1	14.66	0.4	0.8	1.5	3.1
T_{21}	BAP 3 + NAA 0.1	11.33	0.3	0.7	1.4	2.9
T_{22}	BAP 1 + NAA 0.5	18.6	1.5	1.8	3.1	3.75
$T2_3$	BAP 2 + NAA 0.5	14.66	0.4	0.7	1.6	3.5
T_{24}	BAP 3 + NAA 0.5	12.00	0.3	0.6	1.5	3.4
T_{25}	Kinetin 1 + NAA 0.01	11.33	0.9	1.2	2.1	4.0
T_{26}	Kinetin 1.5 + NAA 0.01	9.66	1.0	1.4	2.2	4.2
T_{27}	Kinetin 2+ NAA 0.01	8.33	1.3	1.6	2.9	3.20
T_{28}	Kinetin 1 + NAA 0.1	13..33	0.9	1.2	1.8	3.8
T_{29}	Kinetin 1.5 + NAA 0.1	9.66	0.7	1.0	2.0	3.9
T_{30}	Kinetin 2 + NAA 0.1	8..66	0.6	0.9	1.9	3.95
T_{31}	Kinetin 1 + NAA 0.5	16.00	0.5	0.8	1.6	2.9
T_{32}	Kinetin 1.5 + NAA 0.5	14.33	0.4	0.7	1.5	3.2
T_{33}	Kinetin 2 + NAA 0.5	11.33	0.2	0.6	1.3	3.5
SE(m)	SE(m)	0.421	0.055	0.057	0.063	0.115
C.D. at 5%	C.D. at 5%	1.193	0.157	0.163	0.179	0.326
C.V.	C.V.	5.905	14.877	10.39	6.07	6.191

Table.2 Number of leaves per explant and number of days taken to shoot initiation

Treatment	Treatment (ppm)	Leaves per plantlet	Days taken to shoot initiation
T_1	BAP 1 + Kinetin 0	3.233	12.33
T_2	BAP 2 + Kinetin 0	4.200	14.33
T_3	BAP 3 + Kinetin 0	3.200	16.66
T_4	BAP 1 + Kinetin 1	4.167	14.33
T_5	BAP 2 + Kinetin 1	4.733	14.00
T_6	BAP 3 + Kinetin 1	4.667	12.66
T_7	BAP 1 + Kinetin 1.5	5.200	15.00
T_8	BAP 2 + Kinetin 1.5	4.167	16.33
T_9	BAP 3 + Kinetin 1.5	4.833	13.66
T_{10}	BAP 1 + Kinetin 2	3.900	14.33
T_{11}	BAP 2 + Kinetin 2	3.200	15.00
T_{12}	BAP 3 + Kinetin 2	4.433	17.66
T_{13}	BAP 0 + Kinetin 1	3.733	12.33
T_{14}	BAP 0 + Kinetin 1.5	4.067	12.00
T_{15}	BAP 0 + Kinetin 2	4.500	13.33
T_{16}	BAP 1 + NAA 0.01	4.467	13.66
T_{17}	BAP 2 + NAA 0.01	4.167	14.66
T_{18}	BAP 3 + NAA 0.01	5.400	16.33
T_{19}	BAP 1 + NAA 0.1	5.633	11.00
T_{20}	BAP 2 + NAA 0.1	4.367	12.66
T_{21}	BAP 3 + NAA 0.1	3.167	15.33
T_{22}	BAP 1 + NAA 0.5	6.433	10.66
$T2_3$	BAP 2 + NAA 0.5	4.567	12.33
T_{24}	BAP 3 + NAA 0.5	3.667	14.00
T_{25}	Kinetin 1 + NAA 0.01	6.833	13.33
T_{26}	Kinetin 1.5 + NAA 0.01	8.067	14.66
T_{27}	Kinetin 2+ NAA 0.01	4.667	15.66
T_{28}	Kinetin 1 + NAA 0.1	4.900	12.00
T_{29}	Kinetin 1.5 + NAA 0.1	4.833	13.33
T_{30}	Kinetin 2 + NAA 0.1	6.167	14.66
T_{31}	Kinetin 1 + NAA 0.5	5.167	9.66
T_{32}	Kinetin 1.5 + NAA 0.5	4.233	11.00
T_{33}	Kinetin 2 + NAA 0.5	5.167	13.33
SE(m)	SE(m)	0.505	0.499
C.D. at 5%	C.D. at 5%	1.428	1.413
C.V.	C.V.	18.714	6.312

When the length of shoot was calibrated 6 weeks after the inoculation the maximum length of shoot was recorded with application of BAP 1ppm + NAA 0.5ppm (1.8 cm) followed by Kinetin 2ppm+ NAA 0.01ppm (1.6 cm), Kinetin 2ppm (1.5 cm), Kinetin

1.5ppm + NAA 0.01ppm(1.4 cm) however it was at par with Kinetin 2 ppm; while the minimum shoot length (0.5 cm) was recorded under BAP 3 ppm + Kinetin 2 ppm

Maximum shoot length (3.1 cm) was recorded 8 weeks after inoculation with application of BAP 1 ppm + NAA 0.5 ppm followed by Kinetin 2 ppm+ NAA 0.01 ppm (2.9 cm), BAP 2 ppm +. Kinetin 2 ppm and Kinetin 1.5 ppm + NAA 0.01 ppm (both the same as 2.2 cm); while the minimum shoot length (0.85 cm) was recorded under BAP 3ppm + Kinetin 2ppm.

The final observation of shoot was recorded 10 weeks after inoculation of explants in to CHU (N6) media, supplemented with the various doses of growth hormones. The maximum shoot length (4.2 cm) was recorded with application of Kinetin 1.5 ppm + NAA 0.01 ppm followed by Kinetin 1 ppm + NAA 0.01 ppm (4 cm), BAP 2 ppm + NAA 0.1 ppm(3.95 cm) and Kinetin 1.5 ppm + NAA 0.1 ppm(3.9 cm); however last two were significantly at par with each other.; while the minimum shoot length (1.56 cm) was recorded with BAP 3 ppm + Kinetin 2 ppm.

Number of shoot per explants

The maximum number of shoot development under *in-vitro* cloning of Papaya were observed in CHU (N6) media supplemented with different concentrations of BAP, Kinetin and NAA. Maximum shoot induction (18.66) was observed within 9 days after inoculation (DAI) in CHU (N6) media supplemented with BAP ppm +NAA 0.5 ppm followed by 1 ppm of BAP + Kinetin 1ppm (18.33), 2 ppm of BAP + Kinetin 1ppm and 1 ppm of BAP + NAA 1ppm (both the same 16.66) while the minimum numbers of shoots (7) were recorded in 3 ppm of BAP + 1.5 ppm of Kinetin.

Leaves per plantlet

The maximum number of leaves per plantlet developed under *in-vitro* cloning of Papaya were observed in CHU (N6) media supplemented with different concentrations of BAP, Kinetin and NAA alone and in combination of each other. Maximum leaves induction (8.670) was observed under Kinetin 1.5 ppm + NAA 0.01 ppm followed by Kinetin 1 ppm + NAA 0.01 ppm(6.833), BAP 1 ppm + NAA 0.5 ppm(6.433) and Kinetin 2 + NAA 0.1 ppm(6.177) while the minimum numbers of leaves (3.167) were recorded in BAP 3 ppm + NAA 0.1 ppm.

Days taken for shoot development

The data presented in the Table 2 clearly shows that how the different doses and combinations of growth hormones influence the development of shoot. When the shoot tip and inflorescence used as explants under *in-vitro* cloning of Papaya c.v. Pusa Delicious the shoot development varied significantly from 9 days to 17 days among the different combination of treatments (Table-4.4 and). The minimum number of days taken for the development. (9days) was recorded with Kinetin 1 + NAA 0.5 ppm (9.66) followed by BAP 1 + NAA 0.5ppm (10.66 days), BAP 1ppm + NAA 0.1ppm and Kinetin 1 ppm + NAA 0.01 ppm. Maximum days taken for shoot development (17.66 days) were recorded in BAP 3 + Kinetin 2ppm which was observed 8 days late from earliest treatment.

In conclusion as it is desirable to get production from each plant in commercial fruit production, Tissue culture technique in Papaya can be boon for the fruit growers all over the world. For the establishment of successful organogenesis in Papaya Tissue Culture, like shoot regeneration and root initiation, the above protocol can be followed for commercial purposes.

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