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Effect of GA_3 and Culture Media on *in vitro* Seed Germination of Papaya cv. TNAU Papaya CO 8

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ABSTRACT: The present investigation was taken up to study the effect of gibberellic acid and tissue culture media on *in vitro* seed germination of papaya to obtain uniform, disease free and healthy seedlings. *In vitro* derived seedlings may serve as explant source for micropropagation in papaya. The experiment was carried out with four treatments replicated four times in two different medium *viz*., MS and WPM. The results of the study revealed that seeds without presoaking and without seed coat inoculated in the Woody plant medium supplemented with 500 ppm GA_3 registered the highest germination percentage (80.40%), seedling length (9.76 cm) and took lesser days for germination (8.23 days). The same treatment in MS medium supplemented with 500 ppm GA_3, also registered lesser days for germination (10.81) and recorded the maximum germination percentage of 72.09% with seedling length of 7.11 cm. Seedling vigour index was recorded maximum of 784.70 and 512.55 in seeds inoculated without presoaking and without seed coat in WPM and MS medium fortified with 500 ppm GA_3. Among the two media used, WPM was observed to be better for *in vitro* germination than MS medium and removal of seed coat also influenced the seed germination faster irrespective of the medium. The *in vitro* generated seedlings can serve as the source of axenic explants for plant regeneration and for further studies.

Keywords: Papaya, *in vitro* seed germination, MS, WPM, GA_3, seed coat.

INTRODUCTION

Carica papaya L. commonly known as papaya or pawpaw, is an important fruit crop belonging to the family, Caricaceae and is widespread all around the tropical and sub tropical regions of the world. Papaya is emerging as a main commercial crop in India, during the last few years because of its high nutritive value and ability to produce fruits throughout the year once it starts flowering. It is also reported as 'universal nutrient basket' and now being exploited for its medicinal and industrial values. Papaya is a wholesome fruit and is rich in vitamin A (2020 IU/100g) and vitamin C, riboflavin and folate. Ripe papaya is relished as a dessert fruit and is also used for preparation of jam, jelly, salads, refreshing drinks, candies, tutti-frutti etc. Unripe fruits are also cooked as vegetable in India. The latex obtained from the unripe fruits is the primary source of the proteolytic enzyme, papain and is used widely in pharmaceutical, beer, cosmetics and leather industry. Papaya is conventionally and commercially propagated by seed (Bhattacharya and Khuspe 2001) and seedling plants of papaya are not genetically uniform and significant variation in fruit yield, quality and disease susceptibility is observed within cultivated populations (Drew, 1988).

TNAU Papaya CO 8 is a dioecious cultivar released by Tamil Nadu Agricultural University during 2011. It is a red pulped cultivar suitable for fresh fruit consumption, papain extraction and processing into value added products. The yield potential of the variety is 200-220 t/ha in a cropping period of 20-22 months and it is tolerant to PRSV at field level when compared to commercially cultivated gynodioecious varieties. The problem associated with cultivation of this variety is its dioecious nature, in which the plants will segregate into female and male plants. In papaya, until flowering the sex of plants cannot be predicted, and hence in dioecious varieties it is recommended to plant 5-6 seedlings per pit and after flowering and identification of sex, only the most vigorous female plants per pit will be retained and the remaining plants will be thinned out. It is also advised to retain one male plant for every fifteen to twenty female plants for effective pollination and good fruit set. Maintenance of 5-6 plants/ pit till flowering in dioecious varieties, results in wastage of inputs. Though, molecular markers to determine the sex of the papaya plants prior to flowering are reported

(Leela *et al*., 2018), but till date usage of molecular markers for sex identification is not commercially exploited in large scale. Hence, clonal propagation will be of great advantage in papaya. From earlier reports, it is understood that vegetative propagation methods *viz*., cutting and grafting has recorded low multiplication rates and therefore, micropropagation represents the only economic way of production of uniform and disease-free planting materials. Papaya clones developed through *in vitro* methods are uniform and true to type (Chan and Teo 2002).

In vitro grown seedlings can be used as explant source, since they are reported to possess high vigour for clonal multiplication in certain varieties of papaya (Efendi, 2017; da Silva, 2014), kiwi fruit (Akbaş *et al.* 2007), dragon fruit (Kari *et al.* 2010), citrus (Hassanein and Azooz 2003), strawberry (Miller, *et al*., 1992) and stone fruits (Şan *et al.,* 2014). In addition, young seedlings are superior to mature shoot tips of papaya because mature tissues are laden with latex, making tissue culture more challenging. Papaya is more susceptible to the viral disease caused by Papaya Ring Spot Virus (PRSV) and *in vitro* seed germination will aid in production of disease free, healthy and uniform plantlets which will serve as a source for explants *i.e.,* micro cuttings for further *in vitro* micropropagation experiments.

MATERIALS AND METHODS

The current study was carried out at the Plant Tissue Culture laboratory, Horticultural College and Research Institute, Coimbatore during 2021-22. The seeds of TNAU Papaya CO 8 were collected from the fruits of sibmated female trees at the College orchard. After discarding the floats, only matured and healthy seeds were selected and were used for the experiment. All the chemicals used in this study were of analytical grade and the growth regulators were of tissue culture grade procured from Hi-media Pvt. Ltd., India and Sigma Aldrich, USA.

Explants sterilization. The papaya seeds were pretreated with 0.1% streptomycin sulphate and 0.5% carbendazim for 15 minutes each. Then the seeds were washed in continuous running tap water and again rinsed for 3 to 4 times with distilled water. Further, seeds were surface sterilized for 30 seconds with 70% ethyl alcohol under laminar air flow chamber. Seeds were then secondary sterilized with 0.1% mercuric chloride for 3-5 minutes followed by three to four times with sterile water wash. Seed coat was then removed using sterilized forceps and needle inside the LAFC.

Culture establishment. Pre-treated and surface sterilized seeds were then inoculated in Murashige and Skoog medium and Woody Plant Medium. The experiment was carried out with four different treatments as detailed below and replicated four times with twenty five explants each.

T_1 - Basal medium + pre-soaked seeds with 500 ppm GA_3 without seed coat

T_2 - Basal medium + pre-soaked seeds with 500 ppm with intact seed coat

T_3 - Basal medium supplemented with 500 ppm GA_3 + seeds without presoaking and without seed coat

T_4 - Basal medium supplemented with 500 ppm GA_3 + seeds without presoaking and with intact seed coat

Cultures were incubated at 25±2°C, 80-85% relative humidity, 16/8 hours of light/dark under a 3000 lux white fluorescent light source and the observations were recorded periodically.

Observations recorded. The observations on the parameters *viz*., germination percentage, days taken for first germination, days taken for 50% germination, seedling height and seedling vigour index were recorded. The seedling height and germination percentage were measured on 30th day after inoculation.

Statistical analysis. The experiment was carried out in completely randomized design. The data were analysed by estimating analysis of variance and working out the critical difference value. The critical difference (CD) values were calculated for five percent probability (0.05) as per the statistical methodologies suggested by Panse and Sukhatme (1976).

RESULTS AND DISCUSSION

Germination Percentage. In the present study, the germination percentage on *in vitro* derived seedlings of papaya was significantly influenced by the treatments as well as by the culture media. It is noticed from the data (Table 1), the treatment T_3 (Basal medium fortified with 500 ppm GA_3 and seeds without presoaking and without seed coat) registered higher germination percentage of 72.09% and 80.40% in MS and WPM respectively. The least germination percentage of 50.45% and 55.90% respectively in MS and WPM was obtained in T_2. The role of GA_3 is to activate cytological enzymes, to increase cell wall flexibility and to improve water absorption and thereby aiding in seed germination more effectively (Anburani and Shakila 2008). GA_3 also encourages shoot elongation in seedlings by enhancing nutrient mobilisation and root activity (Barche *et al*., 2008). Earlier workers reported that *in vitro* and *in vivo* seed germination was affected by seed coat removal (Shankarraja and Sulikeri 1993; Page and Staden, 1985; Kyauk *et al*., 1995; Tseng, 1991) and in particular, seed coat removal has fastened *in vitro* germination in papaya (Bowiya *et al*., 2019). The maximum germination percentage observed in WPM compared to MS, might due to low concentration of NH_4NO_3 and replacement of K_2SO_4 instead of KNO_3 in WPM. Highest seed germination percentage in woody plant medium might also be due to lesser nitrogen content present in the WPM medium and similar findings are reported by Xiaoli *et al*. (2012).

Days taken for first germination and 50% of germination. In the present study, the days taken for first germination and 50% of germination were significantly influenced by the treatments as well as by the culture media. The days taken for first germination ranged from 8.23 to 22.98 irrespective of the culture medium. Among the treatments, T_3 (Basal medium fortified with 500 ppm GA_3 and seeds without presoaking and without seed coat) recorded least days for first germination of 10.81 and 8.23 days in MS

medium and WPM respectively (Table 2). Longer days for first germination (22.98 and 21.20) were observed in treatment T_2 in MS medium and WPM respectively. Similar results were also obtained for days to 50% of germination of papaya seeds. The seeds devoid of seed coat exhibited earlier germination compared to seeds inoculated along with seed coat. Seed coat removal enabled earlier attainment of first germination and fifty percent germination. These results are in line with the findings of Bhattacharya and Khuspe (2001). In addition, seed germination in the treatment T_3 was improved due to the involvement of GA_3 by activation of cytological enzymes, increasing cell wall plasticity and better water absorption (Anburani and Shakila 2008).

Seedling height and seedling vigour index. The *in vitro* seedlings of TNAU Papaya CO 8 attained 9.76 cm height at 30 days after inoculation in the treatment T_3 and lowest seedling height was recorded in T_2 (5.64 cm) in WPM medium (Table 3). While in MS medium, the maximum height of *in vitro* seedlings was recorded in T_3 (7.11 cm) and the lowest seedling height (4.78 cm) was registered in T_2 (Table 3). The seedling vigour index was calculated in the present study and the highest seedling vigour index was recorded in WPM medium in T_3 (784.70) and lowest seedling vigour index in T_2 (315.28). In MS medium maximum seedling vigour index was reported in T_3 (512.55) and minimum seedling vigour index recorded in T_2 (241.51). The maximum seedling height was recorded in WPM compared to MS medium and it may due to presence of potassium sulphate in the WPM medium. Similar finding that the influence of potassium sulphate in seedling growth and high seedling vigour index was reported by Aliloo (2015).

Table 1: Effect of different treatments on *in vitro* germination percentage in papaya.

Treatments		MS	WPM
T_1	Basal medium + pre-soaked seeds with 500 ppm GA_3 without seed coat	56.75 (48.90)	63.38 (52.79)
T_2	Basal medium + pre-soaked seeds with 500 ppm with intact seed coat	50.45 (45.28)	55.90 (48.41)
T_3	Basal medium supplemented with 500 ppm GA_3 + seeds without presoaking and without seed coat	72.09 (58.14)	80.40 (63.75)
T_4	Basal medium supplemented with 500 ppm GA_3 + seeds without presoaking and with intact seed coat	68.66 (55.99)	66.30 (54.54)
SE d		**2.03**	**1.79**
CD (p=0.05)		**4.42***	**3.89***
CV (%)		**4.62**	**3.80**

*Significant at 5% level of significance

Table 2: Effect of different treatments on days taken for germination and 50% germination in papaya.

Treatments		Days taken for germination		Days taken to 50% of germination	
		MS	WPM	MS	WPM
T_1	Basal medium + pre-soaked seeds with 500 ppm GA_3 without seed coat	15.14	14.52	20.86	19.16
T_2	Basal medium + pre-soaked seeds with 500 ppm with intact seed coat	17.28	15.76	22.98	21.20
T_3	Basal medium supplemented with 500 ppm GA_3 + seeds without presoaking and without seed coat	10.81	8.23	15.92	14.69
T_4	Basal medium supplemented with 500 ppm GA_3 + seeds without presoaking and with intact seed coat	13.99	11.46	18.82	16.20
SE d		**0.52**	**0.48**	**0.27**	**0.58**
CD (p=0.05)		**1.13***	**1.05***	**0.58***	**1.27***
CV (%)		**5.10**	**5.47**	**1.94**	**4.69**

*Significant at 5% level significance

Table 3: Effect of different treatments on seedling height and seedling vigour index in papaya.

Treatments		Seedling height		Seedling vigour index	
		MS	WPM	MS	WPM
T_1	Basal medium + pre-soaked seeds with 500 ppm GA_3 without seed coat	5.99	6.13	339.93	388.52
T_2	Basal medium + pre-soaked seeds with 500 ppm with intact seed coat	4.78	5.64	241.51	315.28
T_3	Basal medium supplemented with 500 ppm GA_3 + seeds without presoaking and without seed coat	7.11	9.76	512.55	784.70
T_4	Basal medium supplemented with 500 ppm GA_3 + seeds without presoaking and with intact seed coat	6.26	8.01	429.81	531.06
SE d		**0.22**	**0.18**	**14.83**	**17.78**
CD (p=0.05)		**0.48***	**0.39***	**32.31***	**38.75***
CV (%)		**5.12**	**3.41**	**5.50**	**4.98**

*Significant at 5% level of significance

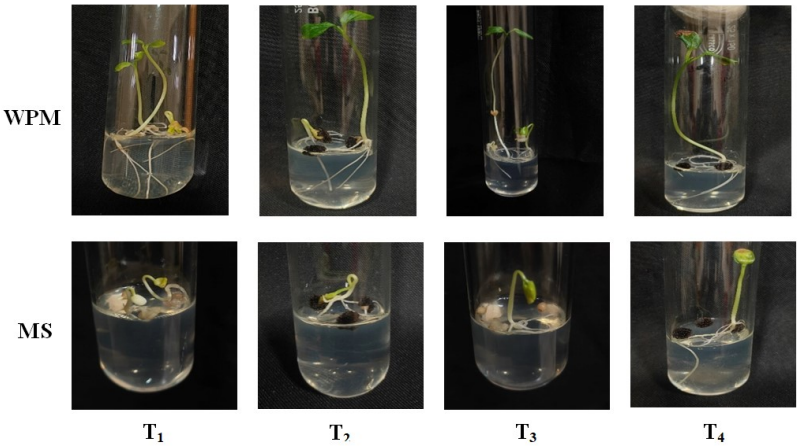

Fig. 1. Effect of GA_3 on culture media on *in vitro* germination of papaya.

CONCLUSION

The experiment was conducted to standardize a protocol for *in vitro* seed germination in papaya. Based on the present study, results concluded that medium supplemented with 500 ppm GA_3 and seeds inoculated without pre-soaking seed coat recorded a maximum germination percentage and highest seedling height in papaya. Lower concentration of inorganic salts in WPM enhanced the seed germination. This technique enables quick and cost-effective production of a large quantity of axenic seedling plants in papaya which can be used for further tissue culture experiments.

FUTURE SCOPE

Further studies are to be carried out for development of larger quantity of *in vitro* seedlings with less time.

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Conflict of Interest. None.

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