DOI : 10.5958/0974-0112.2022.00065.2

Indian J. Hortic. 79(4), December 2022: 464-470

A comparative evaluation of micro-propagated and seed derived plants of intergeneric papaya hybrids

Kaluram*, Vasugi C., Pious Thomas, M. R. Dinesh and Nandeesha P.****

Division of Fruit crops, Indian Institute of Horticultural Research, Hesaraghatta Lake, Bangaluru-560089, Karnataka, India.

ABSTRACT

As papaya is susceptible to papaya ring spot virus (PRSV) disease, it is a challenging task to obtain the true-to-type plants with virus resistance. Hence, a comparative study using morphological traits, fruit traits and above all, resistance to PRSV of field established micro-propagated and seedling derived plants of intergeneric hybrid (IGH) (*Carica papaya* cv. Arka Surya × *Vasconcellea cauliflora* L.) was conducted to obtain virus resistant plants coupled with desired fruit traits. Plantlets were raised using shoot tips of seeding plants from selected advanced intergeneric papaya hybrids (IGH 4-13 and IGH 12-2) on MS media supplemented with 3% sucrose + BAP 1µM + GA3 1µM + IAA 0.1 µM; phytagel / gelrich 0.25%. Intergeneric hybrid plants (micro-propagated and seedling) were field planted and evaluated for sex determination, morphological traits, fruit traits and PRSV resistance. Micro-propagated IGH plants showed significant morphogenic difference, when compared to plants raised through seed like dwarf stature, bearing fruits lower on the stem, flowering at an early time and lower stem circumference, internode length and number of leaves. Among the tissue culture plants, derived from the same mother culture uniformity with respect to morphological, fruit, yield and PRSV tolerance were obtained indicating the identical production of quality plants as the mother culture. Based on the final scoring for PRSV tolerance, the micro-propagated plants *viz*., IGH.SD.3 and IGH.SD.4 showed field tolerance to PRSV coupled with desirable fruit traits. This evaluation study can facilitate breeders and growers to have disease-resistant papaya plants with desired fruit trait.

Key words: *Carica papaya* L*,* GIH, mother culture, PRSV

INTRODUCTION

Papaya (*Carica papaya* L., *Caricaceae*) is one of the most important fruit crops of the tropical and subtropical regions of the world. The main producers include India, Dominican Republic, Brazil and Mexico (FAO, 9). The major papaya producing states are Andhra Pradesh, Gujarat, Karnataka, Madhya Pradesh, Maharashtra, Chhatisgarh, West Bengal, Assam, Tamil Nadu, Jharkhand and Uttar Pradesh (NHB, 16). In the Indian subcontinent, the papaya is grown commercially through seeds (Bhattacharya and Khuspe, 2), and the vegetative propagation practices such as layering, grafting and rooting of cuttings (Soomark and Tai, 19) have not resulted in effective mass proliferation of hermaphrodite papaya.

The limitation is the segregation to male and female in dioecious types, and female and hermaphrodite in gynodioecious types. The species has three sex types: staminate, pistillate and hermaphrodite (Dinesh *et al*., 6). Another biggest limiting factor for papaya cultivation in world over is the Papaya Ring Spot Virus (PRSV) causing 70-80% loss in plantations (Patil *et al*., 18). The annual production is also hampered by this viral disease, and breeders are trying to yield resistant variety (Bhattacharya and Khuspe, 2). The most effective way to address the disease problem is the use of resistant varieties. At ICAR-IIHR, Bengaluru, the intergeneric hybrids (IGH) showed field tolerance to PRSV coupled with desirable fruit traits. The intergeneric progenies were selected at F_8 and F_9 generation, and a few promising lines were identified for micro-propagation. In order to go for large scale multiplication of the identified progenies by vegetative means, micro-propagation protocol has been attempted for the generation of uniform plants. The micro-propagated plants are generated from shoot tips of intergeneric seedling plants, because it is difficult to initiate cultures from field grown plants (Thomas and Kumari, 21) due to high contamination.

Very scanty information is available regarding field presentation of *in vitro* raised plants in comparison of seedling plants, both in terms of morphology and fruit aspects (Pandey and Singh, 17). The real applicability of tissue culture plants would ultimately depend on the comparative field presentation with those of seed grown plants (Zaman *et al*., 23). No

*Corresponding author: kriari25@gmail.com

**Division of Biotechnology, IIHR, Hesaraghatta Lake, Bangaluru-560089, India

literature is available on the field presentations of micro-propagated intergeneric hybrid papaya plants in India. So the present investigation was, therefore, undertaken to determine the relative performances in terms of morphological, fruit as well as PRSV resistant aspects on tissue culture IGH and those obtained through seeds under field condition.

MATERIALS AND METHODS

The experiments were conducted with papaya ring spot virus tolerance advanced generation intergeneric hybrid (IGH) of papaya developed at ICAR-Indian Institute of Horticultural Research, Bengaluru during the period of 2017-2020. The advanced generation intergeneric hybrids (F_8 and F_9) developed from the cross *C. papaya* cv. Arka Surya × *V. cauliflora* L. The original cross performed by Dinesh *et al*. (7), and nine generations of sole plant selection was done by selfing the hermaphrodite tolerant offspring (Dinesh *et al*., 8). It is gynodioecious, having medium size fruits (600g to 1200g) with TSS (9-11 ºBrix), and coupled with field tolerance to PRSV.

The experiment was laid out with micro-propagated and seed derived IGH plants in randomized block design. Forty-five days old vigorous seedlings of IGH (25 Plants) were transplanted along with 117 plants of micro-propagated IGH in the field at a distance of 2m × 2 m. Regular package of practices were followed during the period of experimentation.

Selfed fruits were collected from the selected advanced intergeneric hybrids (IGH 4-13 and IGH 12-2), and seeds were extracted from the ripe fruits, sown in the pro-trays after treating with 100 ppm GA_3. The shoot tips from the 14-15 days old seedlings were used as explants for *in vitro* multiplication. Plantlets were raised by culturing shoot tip explants on MS media (Murashige and Skoog, 15) supplemented with 3% sucrose + BAP 1µM + GA_3 1µM + IAA 0.1 µM; phytagel / gelrich 0.25% was used. These plantlets were taken out of the culture vessels and after thorough washing to remove the remains of the media, were planted in polybags containing non–sterile perlite + vermiculite + coco peat (1:1:2 ratio). For the first one month the polybags were kept inside the growth chamber having fluorescent tubes 20 W daylight lamps (3000 lux) and afterward they were transferred in the poly house for hardening. Finally acclimatized plants were planted in the field along with seedling plants. A total of 39 mother cultures were initiated and labeled as IGH.SD.1 to IGH. SD.39. Three plants from each IGH mother cultures were generated totaling to 117 plants. Hence, a total of 142 plants consisting of 25 plants of seedling origin and 117 plants of IGH micro propagated were field planted and evaluated for sex differentiation, morphological traits, fruit traits and PRSV resistance. For morphological evaluation, parameters like, height of plants (cm), stem circumference (cm), internode length (cm), number of leaves, days to first flowering, height to first fruiting (cm), yield (no. of fruits)/ plant and incidence of PRSV were considered. The disease intensity was recorded using the scale given by Dhanam (5). The scale comprises of five levels [resistant (0-1), tolerant (1-2), moderately susceptible (2-3), susceptible (3-4) and highly susceptible (4 and above)] based on the symptoms displayed by the diseased plants.

To compare the micro-propagated plants with the seedling plants, seedlings of selected IGH were raised in polythene bags packed with soil, sand, farm yard manure and cocopeat in 1:1:1:1 ratio. Germination started in about 10 to 12 days after sowing. Proper watering and plant safety measures were taken up for the seedlings and were planted in the field after 45 days of sowing. PCR (polymerase chain reaction) was done using primers T_1, T_{12} and W_{11} (Deputy *et al*., 4) for sex identification before transplanting in the main field.

Data on days to first flowering and height to first fruiting was recorded at first flowering and at first fruiting, respectively. The paired 't' test was applied to test the significance of field performance between *in vitro* and seed grown plants. Also, basic statistics was performed to evaluate the progeny performance.

RESULTS AND DISCUSSION

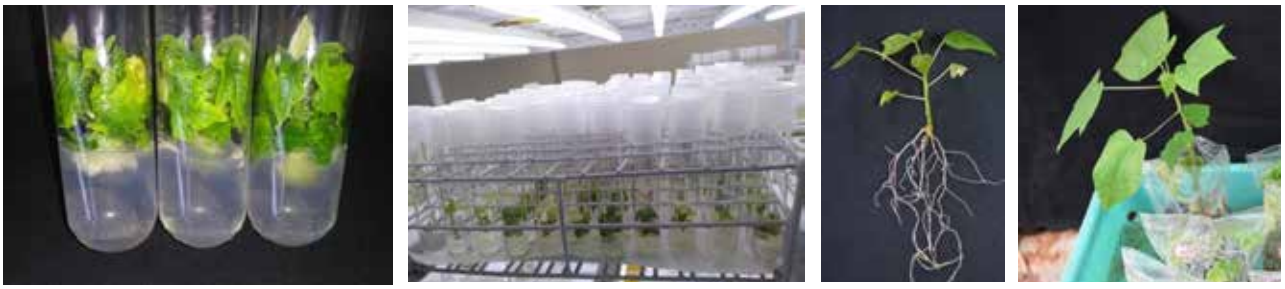
The cultures were established on MS media (Fig.1), and plantlets were taken for *ex vitro* rooting using IBA, phloroglucinol and endophytic bacteria. Plantlets were rooted *ex vitro* and established in the field. There were no phenotypic variants among the tissue culture plants in the field trials. PCR screening of 142 plants for sex appearance showed same result with regard to the expression of sex. Hermaphrodites formed amplified bands at 1300 and 800bp and were recognized by the 800bp band that was missing in the females (Kaluram *et al*., 14).After completion of one year both micro-propagated and seed derived plants showed different morphological characters. The data on comparative field performance of *in vitro* raised and seed grown plants in relation to different growth variables are summarized in Table 1 and 2.

Morphological characters of both micro-propagated and seed derived plants of IGH papaya showed significant differences except number of fruits per plant (Table 3).Maximum height (199.00 cm) was recorded in seedling plants of IGH P-4 whereas; in *in vitro* raised plants of the same genotype the value

Table 1. Morphological traits of the intergeneric hybrid progenies of micro propagated origin.

Progeny	Plant height (cm)	Stem circumference (cm)	Length of internodes (cm)	Number of leaves at fruit maturity	Days to first flower emergence	Height to first fruiting (cm)	Number of fruits per plant	PRSV incidence (1 to 5 score) After 1 year
IGH.SD.1	141.80	20.40	5.00	11.60	98.00	94.20	17.00	5
IGH.SD.2	147.00	24.70	5.00	17.30	109.60	92.30	27.33	4
IGH.SD.3	149.50	20.00	5.00	22.50	86.00	76.50	43.50	2
IGH.SD.4	150.30	25.00	5.00	14.60	107.60	88.30	48.00	2
IGH.SD.5	113.60	21.00	4.00	12.40	112.80	76.20	15.80	5
IGH.SD.6	141.20	22.40	5.00	17.40	97.80	86.80	39.80	4
IGH.SD.7	138.60	21.20	4.60	16.40	102.00	74.00	39.80	4
IGH.SD.9	138.80	23.00	4.60	16.20	108.80	86.00	17.80	4
IGH.SD.10	118.20	20.70	4.00	15.20	93.00	83.20	25.25	4
IGH.SD.14	141.30	23.70	4.60	10.00	105.00	93.00	11.00	5
IGH.SD.15	140.00	24.50	4.50	16.50	103.50	84.00	16.50	4
IGH.SD.16	143.60	23.00	5.00	19.30	97.30	87.30	16.00	4
IGH.SD.17	123.50	20.00	4.00	13.50	105.00	85.50	43.75	4
IGH.SD.18	141.20	22.00	4.70	21.70	103.50	94.70	14.25	4
IGH.SD.19	133.70	20.70	4.50	10.50	104.50	82.70	17.00	4
IGH.SD.21	127.20	21.20	4.20	11.20	110.80	85.60	10.40	5
IGH.SD.22	135.60	23.20	4.60	12.60	108.60	81.60	13.40	5
IGH.SD.23	112.40	19.00	4.00	17.60	80.00	60.80	16.60	4
IGH.SD.25	114.00	21.00	4.20	12.40	90.00	89.40	19.60	4
IGH.SD.26	118.00	21.20	4.20	11.20	110.60	79.00	15.80	4
IGH.SD.27	121.00	21.50	4.20	16.00	87.50	79.70	18.50	4
IGH.SD.30	138.70	24.70	5.00	17.50	109.70	70.50	23.25	4
IGH.SD.31	128.00	20.60	4.20	10.40	103.40	91.20	10.40	5
IGH.SD.32	122.50	19.50	4.00	10.50	112.00	82.50	15.00	4
IGH.SD.33	130.00	21.00	4.40	11.60	95.20	86.20	11.20	5
IGH.SD.34	130.00	20.40	4.40	10.70	107.50	83.20	10.50	5
IGH.SD.35	138.00	24.00	4.60	20.80	90.20	83.00	10.00	5
IGH.SD.36	131.00	23.00	4.40	16.60	105.80	89.00	10.00	5
IGH.SD.37	127.00	22.20	4.60	11.00	95.00	80.00	10.00	5
IGH.SD.38	128.00	21.20	4.40	9.40	110.00	83.80	17.00	4
IGH.SD.39	144.00	25.20	4.80	15.40	102.40	99.40	10.00	5
AS	175.00	29.10	5.00	20.00	124.00	120.00	43.00	5
VC	152.00	24.80	4.00	16.00	130.00	108.00	20.00	1
Mean	134.39	22.28	4.51	14.74	103.25	86.00	20.53	
SEm±	2.30	0.37	0.06	0.64	1.80	1.82	2.02	
CV (%)	9.85	9.60	8.02	25.08	10.02	12.18	56.73	

Note: PRSV Score: 0-1-Resistant, 1-2-Tolerant, 2-3-Moderately susceptible, 3-4-Susceptible & 4 and above-Highly susceptible. IGH-Intergeneric hybrid, SD- Seedling (micro propagated), AS-Arka Surya, VC-*Vasconcellea cauliflora*.



In vitro IGH cultures | Incubation chamber | Rooted plant | Hardened plant

Fig. 1. *In vitro* cultures, *ex vitro* rooted and harden plantlets of IGH.

Table 2. Morphological traits of the intergeneric hybrid progenies of seedling origin.

Progeny	Plant height (cm)	Stem circumference (cm)	Length of internodes (cm)	Number of leaves at fruit maturity	Days to first flower emergence	Height to first fruiting (cm)	Number of fruits per plant	PRSV incidence (1 to 5 score) After 1 year
IGH P-1	195.00	32.00	5.00	19.00	131.00	110.00	40.00	4
IGH P-2	180.00	28.00	5.00	14.00	151.00	110.00	26.00	4
IGH P-3	198.00	27.00	5.00	25.00	150.00	110.00	41.00	4
IGH P-4	199.00	29.00	5.00	22.00	147.00	110.00	39.00	4
IGH P-5	160.00	15.00	5.00	12.00	140.00	100.00	10.00	5
IGH P-6	154.00	14.00	5.00	11.00	138.00	105.00	15.00	5
IGH P-7	180.00	22.00	5.00	14.00	151.00	115.00	15.00	5
IGH P-8	192.00	24.00	5.00	15.00	112.00	123.00	13.00	5
IGH P-9	175.00	19.00	5.00	12.00	146.00	120.00	17.00	4
IGH P-10	178.00	27.00	5.00	16.00	152.00	100.00	30.00	4
IGH P-11	198.00	34.00	5.00	25.00	150.00	102.00	42.00	4
IGH P-12	190.00	29.00	5.00	19.00	150.00	105.00	30.00	4
IGH P-13	170.00	27.00	5.00	17.00	150.00	100.00	27.00	4
IGH P-14	180.00	28.00	5.00	17.00	108.00	120.00	10.00	5
IGH P-15	170.00	26.00	5.00	14.00	140.00	110.00	15.00	5
IGH P-16	195.00	24.00	5.00	15.00	133.00	103.00	13.00	5
IGH P-17	170.00	26.00	5.00	15.00	129.00	115.00	18.00	4
IGH P-18	198.00	31.00	5.00	16.00	117.00	110.00	15.00	5
IGH P-19	158.00	21.00	5.00	17.00	151.00	116.00	12.00	5
IGH P-20	156.00	22.00	5.00	18.00	148.00	112.00	20.00	4
IGH P-21	160.00	24.00	5.00	18.00	134.00	110.00	10.00	5
IGH P-22	180.00	27.00	5.00	18.00	140.00	106.00	25.00	4
IGH P-23	160.00	21.00	5.00	16.00	148.00	105.00	10.00	5
AS	175.00	29.10	5.00	20.00	124.00	120.00	43.00	5
VC	152.00	24.80	4.00	16.00	130.00	108.00	20.00	1
Mean	176.92	25.23	4.96	16.84	138.80	109.80	22.24	
SEm±	3.12	0.96	0.04	0.70	2.60	1.33	2.26	
CV (%)	8.83	19.13	4.03	21.04	9.37	6.06	50.89	

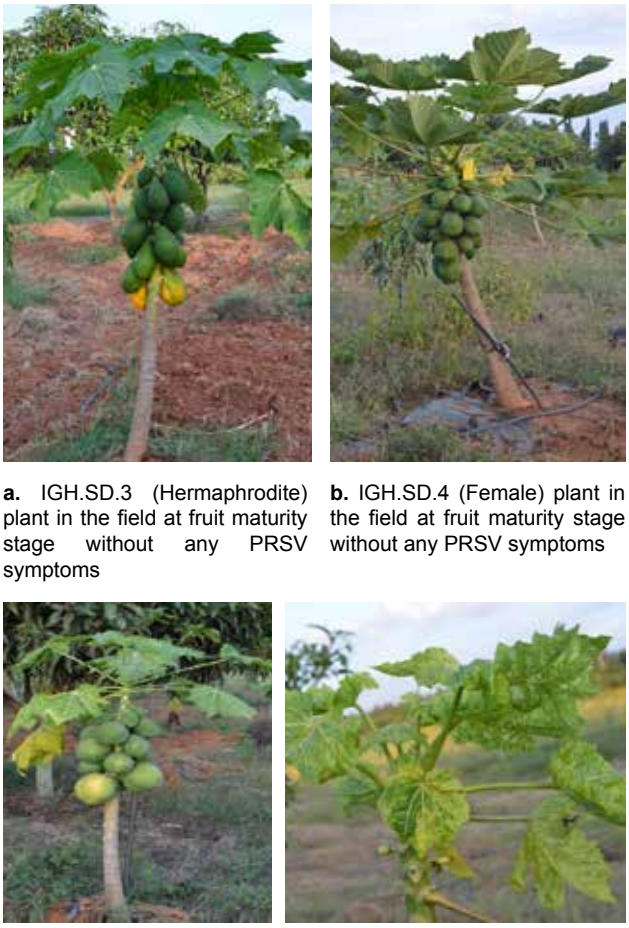
Note: PRSV Score: 0-1-Resistant, 1-2-Tolerant, 2-3-Moderately susceptible, 3-4-Susceptible & 4 and above-Highly susceptible. IGH-Intergeneric hybrid, P-Plant, AS-Arka Surya, VC-*Vasconcellea cauliflora* L.

Table 3. Comparison of morphological and fruit trait of intergeneric hybrids of micro-propagated and seedling origin.

Source of variation	Plant height (cm)	Trunk circumference (cm)	Internode length (cm)	Number of leaves at fruit maturity	Days to first flower emergence	Height to first fruiting (cm)	Number of fruits per plant
IGH TC plants	134.38*	22.27*	4.50*	14.72*	103.24*	85.98*	20.52 ^{NS}
IGH seedling	176.92	25.23	4.96	16.84	138.80	109.80	22.24

(NS) Nonsignificant and (*) significant at $P<0.05$

was 150.30 cm (IGH.SD.4). Similarly, Araya-Valverde *et al*. (1) reported that tissue culture plants had lower plant height than seedling plants of papaya. The plant internode length was reported higher in seedling plants than *in vitro* raised plants. The similar findings were also reported by Gatambia *et al*. (11). Since tissue cultured plants had shorter internodes than the seedling plants which proved dwarfism. Storey (20) reported that tree stature and height of initial flowering is determined by length of internode. Because of lower flowering height tissue cultured plants had lower first fruiting height also as observed with a range from 60.80 cm (IGH.SD.23) to 99.40 cm (IGH.SD.39) compared to seedling plants which ranged from 100.00 cm (IGH P-5, 10 and 13) to 123.00 cm (IGH P-8) (Fig. 2c). The stem circumference was higher in seedling plants than *in vitro* raised plants. In the direct seeded plants, IGH P-11 had the highest mean circumference of 34.00 cm followed by 25.20 cm in tissue cultured papaya of IGH.SD.39. According to Pandey and Singh (17) and Gatambia *et al*. (11), a significant difference were found in stem circumference between *in vitro* raised and seed derived plants of papaya. Seedling plants of IGH had higher number of leaves than micro-propagated plants. Contradictory findings to this have also been reported by Pandey and Singh (17) in papaya. Micro-propagated papaya plants took a shorter time for blossoming than their counterpart's seedling plants. These shoot tip cultured papaya took short duration i.e.112.80 days (IGH.SD.5) to blossoming, while seedling plants took longest duration 152.00 days (IGH P-10). Early flowering in shoot tip cultured plants showed influence of the juvenility factor which continues longer in seedlings. Similar results were reported by Fitch *et al*. (10). Clonally propagated plants developed flowers earlier compared to seedlings which are also supported by the work of Pandey and Singh (17).There were no statistical differences in yield expressed as number of fruits per plant within a year (Table 3).The higher number of fruits (48.00) was recorded in IGH.SD.4 tissue cultured plants than IGH P-11(42.00) seedling plants. Similar results were also reported by Chan and Teo, (3) and Fitch *et al*. (10). In the present investigation, PRSV scoring was done during vegetative, blossoming, fruiting and at picking phases at monthly intervals among the tissue cultured and seedling IGH plants, which showed variable level of disease frequency. Based on the last counting at the time of harvest, IGH.SD.3 and IGH.SD.4 showed field tolerance (Fig. 2a and b), while all the IGH seedling plants expressed susceptibility (Fig. 2d). This is in



a. IGH.SD.3 (Hermaphrodite) plant in the field at fruit maturity stage without any PRSV symptoms

b. IGH.SD.4 (Female) plant in the field at fruit maturity stage without any PRSV symptoms

c. IGH tissue culture plant with short height

d. PRSV infected IGH seedling plant in the field

Fig. 2. IGH.SD.3 and 4 plants in the field at fruit maturity stage without any PRSV symptoms, tissue cultured plant having dwarf stature and seedling plant with PRSV symptoms.

agreement with the outcomes of Dhanam (5) and Jayavalli *et al*. (12) were found disease free plants in the F_2 progenies of intergeneric origin with *V. cauliflora* L. as male parent. Tissue culture planting materials were found to be virus free (PRSV) as confirmed by Villegas and Valencia (22).

Fruit parameters did not differ significantly among the seedling derived and tissue cultured plants. The total soluble solids of fruits were in the range of 9.86 °Brix to 11.86 °Brix in tissue cultured plants. The TSS is one of the traits, which determines the overall fruit quality. As the wild species used in the current study was of poor quality (Jimenez and Horovitz, 13). There were ample risks for receiving poor quality fruits in the subsequent hybrids. In our research, we observed that IGH clones from same mother plant and even seedling plants showed same range of TSS. Similar findings were also reported by Chan and Teo (3).

In conclusion, using F_8 and F_9 intergeneric hybrid to get tolerance/ resistant cultivar, we have successfully generated and evaluated tissue culture plants from shoot tips of seedling and obtained two tissue cultured plants (IGH.SD.3 and IGH.SD.4) field tolerance to PRSV disease. The both progenies have horticultural properties most similar to the mother cultures. Furthermore, tolerance plants to PRSV can be generated through *in vitro* cultures of both the identified plants. The major characteristics of the micro-propagated IGH papaya as compared to seed propagated one were as follows: Dwarf stature, lower stem circumference, shorter internodes, lower leaves, early flowering and lower fruiting height whereas yield and fruit traits were within the normal range. Therefore, we expect that the tissue culture method will be applicable in commercial production of virus free papaya plants with stable fruit quality in other papaya cultivars.

AUTHORS' CONTRIBUTIONS

Kaluram and Vasugi C contributed to data acquisition and wrote the manuscript. MR Dinesh and Nandeesha P participated in clarification of data and reviewing the manuscript for intelligent content. Pious Thomas made considerable contributions to the commencement and strategy of the study, elucidation of data, and rereading the manuscript for intellectual content.

DECLARATION

The authors declare that there is no conflict of interest

ACKNOWLEDGEMENT

I wish to acknowledge and thank director, dean and faculty members of division of fruit science, IIHR, Bengaluru and IARI, New Delhi for providing me the opportunity to do my Ph.D. programme at this institute and this work was supported by the University Grants Commission (UGC) of Ministry of Education, New Delhi.

REFERENCES

1. Araya-Valverde, E., Bogantes, A., Holst, A., Vargas-Mora, C., Gómez-Alpízar, L., Brenes, A., Sánchez-Barrantes, E., Chavarría, M. and Barboza-Barquero, L. 2019. Field performance of hermaphrodite papaya plants obtained through molecular selection and micro-propagation. *Crop Breed. Appl. Biotechnol*. **19**: 420-27.

2. Bhattacharya, J. and Khuspe, S. S. 2001. *In vitro* and *in vivo* germination of papaya (*Carica papaya* L.) seeds. *Sci. Hortic*. **91**: 39-49.

3. Chan, L. K. and Teo, C. K. H. 2002. Micro-propagation of Eksotika, a Malaysian papaya cultivar, and the field performance of the tissue culture derived clones. *Acta Hortic*. **575**: 99-105.

4. Deputy, J. C., Ming, R., Ma, H., Liu, Z., Fitch, M., Wang, M., Manshardt, R. and Stiles, J. L. 2002. Molecular markers for sex determination in papaya (*Carica papaya* L.). *Theor. Appl. Genet*. **106**: 107-11.

5. Dhanam, S. 2006. Studies on papaya ring spot disease, *M.Sc. Thesis*, Tamil Nadu Agricultural University, Coimbatore.

6. Dinesh, M. R., Reddy, B. M. C. and Reena, N. A. 2001. Varietal improvement of papaya (*Carica papaya* L.). *J. Appl. Hortic*. **2**: 121-23.

7. Dinesh, M. R., Rekha, A., Ravishankar, K. V., Praveen, K. S. and Santosh, L. C. 2007. Breaking the intergeneric crossing barrier in papaya using sucrose treatment. *Sci. Hortic*. **114**: 33-36.

8. Dinesh, M. R., Veena, G. L., Vasugi, C., Reddy, M. K. and Ravishankar, K. V. 2013. Intergeneric hybridization in papaya for 'PRSV'tolerance. *Sci. Hortic*. **161**: 357-60.

9. FAO (2019).FAOSTAT database collections. http://faostat.fao.org

10. Fitch, M. M., Moore, P. H., Leong, T. C., Akashi, L. A. Y., Yeh, A. K., White, S. A. and Poland, L. J. 2005. Clonally propagated and seed-derived papaya orchards: I. Plant production and field growth. *HortSci*. **40**: 1283-90.

11. Gatambia, E. K., Kihurani, A. W., Rimberia, F. K. and Waiganjo, M. M. 2016. Evaluation of growth rate and phenotypic traits of meristem-cultured papaya plants. *J. Exp. Agric. Intl*. **14**: 1-8.

12. Jayavalli, R., Balamohan, T. N., Manivannan, N., Rabindran, R., Paramaguru, P. and Robin, R. 2015. Transmission of resistance to papaya ringspot virus (PRSV) in intergeneric populations of *Carica papaya* and *Vasconcellea cauliflora*. *Sci. Hortic*. **187**: 10-14.

13. Jimenez, H. and Horovitz, S. 1957. Cruzabilidad entree species de Carica. *Agronomia Trop.* **7**: 207–15.

14. Kaluram, Vasugi, C., Thomas, P., Dinesh, M.R. and Nandeesha, P. 2022. Sex determination of papaya plants derived from seed and micro-propagation. *Indian J. Hortic*. **79**: 3-8.

15. Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiol. Plant*. **15**: 473-97.

16. NHB 2018. *Year book of the National Horticultural Board*, GOI, 2017-18.

17. Pandey, R. M. and Singh, S. P. 1988. Field performance of *in vitro* raised papaya plants. *Ind. J. Hortic*. **45**: 1-7.

18. Patil, P., Vastrad, N., Dinesh, M. R. and Bantwal, A. R. 2007. A revised protocol for *in -vitro* propagation of *Carica papaya* using lateral buds from field-grown trees. *J. Hortic. Sci*. **2**: 99-103.

19. Soomark, S. and Tai, E. A. 1975. Vegetative propagation of papaya by budding. *Acta Hortic*. **49**: 85-90.

20. Storey, W. B. 1953. Genetics of the papaya. *J. Hered.* **44**: 70-78.

21. Thomas, P. and Kumari, S. 2010. Inconspicuous endophytic bacteria mimicking latex exudates in shoot-tip cultures of papaya. *Sci. Hortic*. **124**: 469-74.

22. Villegas, V. N. and Valencia, L. D. 1998. Comparative performance of micro-propagated and conventionally seed-derived Sinta' hybrid papaya. *Philipp J. Crop Sci*. **23**: 13-19.

23. Zaman, A., Islam, R. and Joarder, O. I. 1997. Field performance and biochemical evaluation of micro-propagated mulberry plants. *PCTOC*. **51**: 61-64.

Received : August, 2022; Revised : October, 2022;
Accepted : November, 2022