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Intra-specific Hybridization in Cultivated Papaya (*Carica papaya* L.) Genotypes

Dorcas Olubunmi Ibitoye* • Pamela Eloho Akin-Idowu

Fruits and Biotechnology Programme, National Horticultural Research Institute, P.M.B. 5432, Ibadan, Nigeria

Corresponding author: *bunmiajisafe@yahoo.com

ABSTRACT

A pioneer breeding study was started at the National Horticultural Research Institute, Ibadan, Nigeria during 2007-2008 on papaya with the aim of addressing the age-long problem associated with seedling sex identification in papaya, which is a major limiting factor in Nigeria for large-scale production of papaya. Morphological and qualitative characters were recorded for the selected parental genotypes and the F_1 progenies. F_1 hermaphrodite plants had lower mean value for plant height than both parents but had higher mean value for stem girth and number of leaves at flowering than one parent. The study revealed that controlled pollination of the hermaphroditic papaya genotypes segregated for female and hermaphrodite progenies at the first filial generation (F_1) while the progenies resulting from male-female crosses segregated for male, female and hermaphrodite genotypes. The highest percentage hybridization success (70.2%) was recorded from selfed hermaphrodites. This result implies that generating more hermaphrodite genotypes under controlled pollination will give desired sex types needed to increase production.

Keywords: filial generation, genotype, hermaphrodite, pollination, progeny

INTRODUCTION

Papaya (*Carica papaya* L.) is a popular and economically important fruit tree of tropical and subtropical countries. The fruit is consumed world-wide as fresh fruit and as a vegetable or used as processed products. Papaya fruits consist mostly of water and carbohydrate, low in calories and rich in natural vitamins and minerals, particularly in vitamins A and C, ascorbic acid and potassium. One hundred g of papaya contains: 55 calories, 0.61 g protein, 9.8 g carbohydrates, 1.8 g dietary fiber, 89% water, 283 IU vitamin A, 62 mg vitamin C, 38 mg folate and 257 mg potassium (USDA 2005).

Papaya is one of the few tree crops that produce ripe fruit as quickly as 9 months from planting. It is polygamous with three sex forms: male, female, and hermaphrodite. Controlled crossing of papaya is easy, using pollen from male or hermaphrodite flowers to fertilize stigmata of female or hermaphrodite flowers. Year-round flowering and fruiting of papaya plants produce a continuous supply of fruit for the evaluation of quality and productivity. The high number of seeds per fruit (about 800–1200), coupled with the perennial fruiting of papaya, can provide virtually unlimited numbers of offspring from a controlled cross. Fruit production may occur following either self-pollination or cross-pollination and is affected by pollinator efficiency or abundance. Honeybees, thrips, hawk moths have been reported as pollinators of papaya (Garrett 1995). Although the floral morphology in papaya plants suggests insect pollination, various authors have indicated that wind pollination may also be important (Nakasone and Paull 1998). The somatic chromosome number in the dicotyledonous genus *Carica*, is 2n=18. Sex determination in papaya has been a frequent subject of genetic analyses (Hofmeyr 1938; Storey 1938; Hofmeyr 1967; Storey 1976) because it is directly related to efficient commercial fruit production. Genetic analysis of papaya sex determination was carried out by crossing individuals of different sex types (Storey 1941). Most *Carica* spp. are dioecious, except for *C. papaya* which is characterized by various flower types and three primary, polygamous sexual types, viz. pistillate (female; mm), staminate (male; M1m) and hermaphrodite (M2m). Intermediate types have also been described (Hofmeyr 1938; Storey 1938, 1953; Chan 1996). Segregation ratios established by the studies in the 1950s showed that males and hermaphrodites are heterozygous, females are homozygous but dominant homozygotes (M_1M_1, M_1M_2, and M_2M_2) are lethal. Lethality is attributed to inert regions missing in M_1 and M_2 (Hofmeyr 1967). Essentially, there are two breeding systems in papaya (Aquilizan 1987; Manshardt 1992): a) The Hawaiian system with truebred lines, e.g. 'Solo', established through inbreeding by pedigree or back-cross breeding; b) the Yarwun (Queensland) system in which homozygous female lines breed with inbred, ambivalent males. In addition to the time-consuming nature of breeding in papaya, in which six generations are needed for homogenization of alleles for a particular trait (Ray 2002), there is also the problem of sex instability.

Interspecific hybridization between cultivated and wild relatives of papaya has been reported by many researchers when trying to breed for quality papaya with relative to pest and disease resistance, frost resistance among many other traits (Muthulakshmi *et al*. 2007). Intraspecific hybridization however is always carried out among related cultivars in order to: (a) make extra genetic gain by combining pairs of desirable traits (Harfouche and Kremer 2000), (b) increase the genetic variation of breeding populations while decreasing the levels of inbreeding (Kaya and Lindgren 1992), (c) utilize hybrid vigour and improve productivity (Harfouche *et al*. 2000) and (d) study quantitative and molecular genetic variation of traits. Papaya trees are grown for fruit that is consumed, and for stems, leaves and roots that have a wide range of applications in folk medicine. In addition, milky juice from unripe papaya fruit is the primary source of papain, a widely used proteolytic enzyme. Papain is widely used in industries like pharmaceutical, food, leather, forage, textile, cosmetic and brewery (Dunne and Horgan 1992). Owing to these vast applications that can be derived from papaya fruit, there is therefore need to increase fruit production by planting papaya as plantation

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Research Note

crops. However, Nigerian farmers do not grow papaya as a plantation crop because of the problem associated with seedling sex identification.

This study therefore, is the first to be embarked on in Nigeria as far as papaya breeding is concerned. Given that the sex of papaya plants cannot be determined for up to 6 months after germination after which many resources in term of time and labour would have been wasted, the establishment of papaya orchards with appropriate sex ratios has been a challenge till today in Nigeria as farmers do not engage in papaya plantation business despite the high demand for fruits and papain derived from the unripe fruits from industries in the country. The aim of this study therefore, is to search for suitable sources of genetic variation from hybridization among existing promising papaya plants from which selection can be made for papaya improvement and towards increasing production of fruit bearing genotypes for increased production in Nigeria harnessing the possibilities that the conventional breeding method can offer. The use of molecular markers for seedling sex identification and use of tissue culture techniques for mass production of desirable sex types is still at its infancy in Nigeria and is being considered to be used in future works.

MATERIALS AND METHODS

The cultivars used were landraces with promising traits and market acceptability. The selection of these cultivars was based on the result of survey that was carried out in Nigeria. The status and some qualitative attributes of these cultivars are provided in **Table 1**.

Seeds were raised in the nursery and later transplanted to the field 6 weeks after at a spacing of 2 m × 2 m with three replications at the experimental field of the National Horticultural Research Institute, Ibadan, Nigeria. The experimental site was a newly cultivated site having a clay-loam soil texture (sand 35.8%, clay 34.9%, silt 29.2%), pH 6.2, extractable acid 0.23, organic matter content 2.2%, Ca 27.7 cmol/L, mg 11.7 cmol/L, K 0.96 cmol/L and a cation exchange capacity of 40.4 cmol/L.

Quantitative and qualitative data recorded in this study were taken at flowering stage of the crop. Data were collected on the following quantitative characters: plant height and girth, number of nodes to first flower, number of functional leaves, length and girth of fruit, fruit weight. Physical observations of the following parameters were also considered and recorded on the selected parental genotypes and their F_1 hybrids: colour of male, female and hermaphrodite flowers, ratio of sex types, percentage hybridization success.

Production of F_1 hybrids

Unopened flowers of identified female parents were covered with pollination bags 7 days before opening, the bag was opened on the 7th day and the petals removed, the exposed stigmatic surface was then dusted with pollen that had been collected from the male donor with the aid of a brush. The flower bud was then re-covered with the pollination bag, tagged and labeled. The hand pollinated flower was then opened five days after in order to ensure that the stigmatic surface had degenerated so as not to receive external pollen thereby avoiding unknown pollination by pollinating agents.

In the case of F_1 hybrids from hermaphrodite parents, the flower buds were covered up without hand pollinating them since the flower has both the male and the female sexes together, the cover was to ensure that the same pollen was responsible for the fertilization of the same stigma so as to produce true-to-type progeny.

Data were collected on the percentage success of the hybridization trial and the ratio of sex segregation at the 1st filial generation.

Data analysis

The data was subjected to analysis of variance (ANOVA) using the generalized linear model (GLM) of SAS (Statistical Analysis System) software version 8.02. (2003).

Means were separated by Duncan's multiple range test (DMRT) and significance level was determined at $P < 0.05$.

RESULTS AND DISCUSSION

Results of the analysis are presented in **Tables 2-4**. The high coefficient of variation observed for stem girth, fruit weight and functional leaves (**Table 2**) indicated that variation among the genotypes for these characters was high and therefore, the traits can serve as indices of selection for yield in a papaya breeding programme while low coefficient of variation in traits like number of nodes to first flower and plant height suggests that the genotypes do not differ much for these traits (**Table 2**).

F_1 population

The sex segregation ratio of the F_1 progenies observed in the crosses attempted in **Table 3** showed that selfing hermaphroditic papaya trees is an indicator to have more female and hermaphrodite sex types, the result of this hybridization agreed with result obtained by Suma (1995) and Hofmeyr (1938).

The F_1 progenies exhibited variability for the morphological characters studied. The F_1 progenies for the hermaphrodite parents were further studied and evaluated for their growth performances (**Table 4**). This was done because of the difficulties in maintaining purity in female plants through crossing and besides, one of the aims of this study is to develop gynodioecious papaya lines.

Generally in papaya, short stature is a desirable character as it helps for easy and early harvesting of fruits. In the present investigation, F_1 progenies had a lower mean plant

Table 1 Morphological characteristics of four papaya cultivars used in this study.

Genotype	Status	Petiole colour	Fruit size	Fruit shape	Flesh colour
NHPS-87-1	Landrace	Purple	Small	Pear-shaped	Orange-red
NHOV-07-1	Landrace	Green	Big	Round	Orange
NHEL-07-02	Landrace	Green	Big	Elongated	Orange
NHJM-87-2	Landrace	Green with tinge of purple	Very big	Round	Yellow

Table 2 Growth and yield parameters of the four selected parental genotypes.

Characters /Genotypes	*NHJM-87-2	NHEL-07-02	NHOV-07-01	NHPS-87-1	Mean ± S.E.	CV (%)
Av. Plant height (cm)	135.10 ± 0.56 b	200.69 ± 0.91a	160.80 ± 0.87 b	198.96 ± 0..85 ab	173.89 ± 12.27	20.14
Av. plant stem girth (cm)	7.78 ± 1.42 ab	10.86 ± 0.85 a	8.17 ± 1.05 a	6.17 ± 1.42 b	8.25 ± 0.19	52.84
Av. No of functional leaves	15.20 ± 0.86 b	20.20 ± 0.91 a	22.20 ± 1.10 a	13.20 ± 0.95 b	17.70 ± 1.8	75.80
Av. No of nodes to first flowering	15.14 ± 1.42 a	21.20 ± 0.94 a	20.20 ± 0.90 a	14.29 ± 0.96 ab	17.71 ± 0.96	19.70
Fruit length (cm)	16.10 ± 1.12 a	12.93 ± 0.77 b	13.67 ± 0.72 b	13.43 ± 0.68 b	14.03 ± 0.23	34.18
Fruit girth (cm)	28.39 ± 0.92 a	25.10 ± 0.96 ab	27.17 ± 1.10 a	27.77 ± 1.05 a	27.10 ± 0.71	31.09
Fruit weight (g)	0.34 ± 1.14 a	0.26 ± 1.10 a	0.33 ± 0.98 a	0.53 ± 1.10 a	0.34 ± 0.06	72.00

*Results are means of three replications ± standard deviations

Values with same letters in the same row are not significantly different at 95% confidence level

Table 3 Sex segregation ratio and hybridization success of the F_1 progenies.

Parent genotypes		Flower colour		Sex segregation ratio for F_1 progenies			% hybridization success
Female	**Male**	**Female**	**Male**	**Male**	**Female**	**Hermaphrodite**	
NHPS-87-1	NHEL-07-02	Yellow	Cream	2	1	1	40.0
NHPS-87-1	NHOV-07-1	Cream	Cream	1	1	-	33.3
NHOV-07-1	NHPS-87-1	Cream	Yellow	1	1	1	50.0
NHEL-07-02	NHPS-87-1	Yellow	Cream	1	1	-	66.7
NHJM-87-2	NHPS-87-1	Yellow	Yellow	1	1	-	51.2
Selfed hermaphrodite genotypes							
NHEL-07-02	NHEL-07-02	Yellow	-	-	1	2	70.2
NHPS-87-1	NHPS-87-1	Cream	-	-	1	2	60.5
NHOV-07-1	NHOV-07-1	Cream	-	-	1	2	68.6

Table 4 Mean performance of parents and progenies for morphological characters.

Characters	NHPS-87-1	NHEL-07-02	Hermaphrodite F_1 plants	NHOV-07-1	NHPS-87-1	Hermaphrodite F_1 plants
Plant height at flowering (m)	198.96	200.69	167.12	160.80	198.96	120.77
Plant stem girth at first flowering (cm)	6.17	10.86	8.50	8.17	6.17	7.82
Number of leaves at first flowering	13.20	20.20	10.55	22.20	13.20	18.55

height at first flowering compared to the parents. The F_1 progenies had high mean stem girth at flowering than the female parent NHPS 87-1.

More number of leaves is a pre-requisite for increased leaf area and increased photosynthesis. In this study, F_1 progenies had a lower number of leaves than both parents in both crosses reported. This result is line with the earlier findings of Kamalkumar (2003).

CONCLUSION

Papaya continues to increase in importance as a fruit crop. This study being a pilot study has revealed the possibility of creating new genotypes among the cultivated varieties in our germplasm through recombination that hybridization offers. It has also shown the possibility of identifying the seedling sex types through the use of conventional breeding technique. Further advancement of the F_1s to F_2 and F_3 generation will give detailed information on the inheritance patterns of genes controlling different desirable agronomic traits in papaya cultivated in Nigeria. This information is very useful in designing breeding technique for the improvement of this useful tropical fruit to increase fruit production that will be adequate both for consumption and industries in need of the fruit latex. Great advantages proffered by tissue culture and use of molecular markers will be explored in the mass production of exact clones of desired sex types and seedling sex identification.

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