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ORIGIN AND EVOLUTION OF FEMALE PLANT FROM AN IDENTICAL MALE PLANT, IN *CARICA PAPAYA* L.

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Abstract

A field study was carried during January 2011 to March, 2013, to confirm the origin and evolution of female plant from an identical male plant in, a dioecious plant, the *Carica papaya* L. The plants were grown from the seeds of a normal female plant fruit. The grown, plants were identified as XX, XY and XYh (in March - April, 2012) on the basis of male and female flower bearing. The identical male plants, which usually bear only male (unisexual) flowers having calyx, corrolla and androecium, were observed also to bear bisexual flower, having calyx, corrolla, and gynoecium (ovary fused with androecium). The fruits were set having the bisexual flowers in the identical male (hermaphrodite) plant. These fruits were kept under observation from setting to ripening stage. The ripened fruits were harvested from the identical male plants and 90-95% fruits from these plants were found with the seeds. Plants grown from these male fruit seeds produced all three type of plants i.e., male, female and hermaphrodite. This study indicated that an identical male (XYh) plant produced the female (XX) plant naturally, because of the XXY= XYh condition, which can contribute basic genetic material to male and female plants i.e an identical male (XYh = XXY= 2N +1 = 18+1= 19) produced all three type of plants, the pure male, the hermaphrodite and the female plant, originated from a single source of an identical male, as shown here. XYh = XXY → XY + XX + XXY. The propagation of all three sexes of *Carica papaya* from a single source of an identical male plant seeds is the first report in the world.

Keywords: Origin, Evolution, Female plant, Identical male, Hermaphrodite, *Carica papaya*.

Introduction

"The hypothesis that angiosperms originated from a hermaphroditic ancestor is supported by the prevalence of hermaphrodites among 72% bisexual flowering species" Takhtajan (1969). Although the vast majority of extant flowering plants are hermaphrodites, monoecious and dioecious species occur in 75% of angiosperm families, including all six dicotyledonous and all five monocotyledonous subclasses (Yampolsky & Yampolsky, 1922; Renner and Ricklefs, 1955). Some dioecious flowering species have an active-Y system of sex determination with heterogametic males (XY) and homogametic females (XX), such as *Carica papaya* and *Asparagus officianalis* (Ono, 1935, Westergaard, 1958).

Papaya is grown in Pakistan, especially in the province of Sindh generally and in the city of Karachi, it is grown commonly not only in rural areas (Gadap town, Memon goth etc.) on a large scale but also commonly cultivated in the gardens of urban areas.

It is reported and hypothesized that an identical male and hermaphrodite papaya plants have the same genotype, based on frequent sex reversals of male-to-hermaphrodite and hermaphrodite-to-male existed (Storey, 1976). Lateral bud culture of papaya for clonal propagation has been reported by Rajeevan & Pandey (1986), whereas in vitro clonal propagation of papaya plant has been reported by Riuveni *et al*., (1990) and Islam *et al*., (1993), while the regeneration of papaya plant through tissue culture was reported by Khatoon & Sultana (1994). Wu *et al*., (2012) demonstrated the propagation of auxiliary and terminal buds from hermaphrodite (identical male) plant using tissue culture. Fujisawa *et al*., (2001) discovered the female sex chromosome from the male sex-chromosomes (XY) from *Marchantia polymorpha* (liverwort). XY-chromosomes system has been reported in White Campion *Silence latifolia* Farbos *et al*., (1999), Lebel-Hardenack *et al*., (2002) and in three spine sticklebacks Peichel *et al*., (2004).

Papaya sex chromosomes have virtually all of the features of human sex- chromosomes. At present, there is no way to determine a plant's sex from its seed. In a typical planting, about two-thirds (66.66%) of the plants are male & hermaphroditic and one-third (33.33%) are female. Once the plants have flowered, workers go through the fields and laboriously remove the males including the hermaphrodites which look similar in appearance to males. Since papayas typically flower between 6 - 12 months of age, farmers have to wait a good while before they could determine the sex. Contrasted with the sex chromosomes of mammals; those of plants have evolved separately in many families and are poorly characterized. In contrast to the roughly 240-320 million year ago of human sex chromosomes, the papaya plant has recently evolved sex-determination system and appears to be at the dawn of sex chromosome evolution. Parallels in the evolution of sex chromosomes in plants and animals are illustrated in that the primitive sex chromosomes in papaya share characteristics that are typical of well developed mammalian sex chromosomes, including suppression of recombination and degeneration of the male sex Y-chromosome (MSY) region, as driving forces in sex chromosome evolution. Genomic analyses of papaya's primitive MSY provide direct evidence that sex chromosomes are evolving from autosomes as predicted by evolutionary biology theory. The male and hermaphrodite papaya contains an allele that is not found in female papaya. Severe recombination suppression and DNA sequence degeneration are observed in the regions around that allele. Although no heteromorphic chromosomes in papaya were found to be analogous to the X and Y-chromosomes in humans, the newly discovered gene shares many characteristics with male-specific region (MSY) of the Y-chromosome (Liu *et al*., 2004).

Papayas have specialized chromosomes that carry genes that determine the gender of their offspring. They are called sex chromosomes and are markedly different from ordinary chromosomes. One particular papaya chromosome resembles a primitive version of the human Y-chromosome. It's thought that a similar process occurred in the human beings (Moore, 2004).

The Y-chromosome is the shortest chromosome in humans, and most of the Y-chromosome is believed to be made of junk DNA. Ohno (1967) proposed that the human Y- chromosome is a profoundly degenerated X-chromosome with very few genes on it encoding male-specific features and some began to speculate that the continued degradation of the Y-chromosome might result in the extinction of the human male. In order to address this question, it is helpful to know the origin of the Y-chromosome. Surprisingly, so far the most primitive Y-chromosome was not found in any animal, but is found in *Carica papaya* (Chen, 2005).

A gradual process of recombination restriction in the evolutionary history of the sex- chromosomes in dioecious plants has been recorded by Nicolas *et al*., (2005). A pistilade (rudimentary ovary) has been reported in the flowers of hermaphrodite plant and the change of sex of the flowers may take place due to age, injury and other treatment. Ten (10) stamens in the male flower of papaya are also reported (Panhwar, 2005). Ming *et al*., (2007a) opined that the dioecious condition had arisen independently from hermaphroditic ancestors in many plant families and genera". Ming *et al*., (2007b) writes that the sequencing of the male-specific region of the Y-chromosome and corresponding region of the X-chromosome is underway in papaya. Some of the other workers have also worked on papaya eg., Sondur *et al*., (1996); Atanassov *et al*., (2001); Ming *et al*., (2001) Matsunaga & Kawano (2001); Chiu (2000); Ma *et al*., (2004); Lai *et al*., (2006); Yu *et al*., (2007, 2008ab, 2009); Zhang *et al*., (2008); Ming *et al*., (2008); Wu *et al*.,(2010); fruits bearing of male *Carica papaya* has been reported by Tariq (2011a), Tariq (2013). In the present work, propagation of three type of plants (pure female, pure male and hermaphrodite), from a single source of an identical male (hermaphrodite) plant, has been proved after the study of three year experiment, for the first time.

Materials and Methods

The seeds of normal female *C. papaya* plants were obtained from large ripe fruits from Gadap Town area, (Kathore). The fruits were brought to the experimental field of Biological Research Centre, University of Karachi. The fruits were opened; the seeds were separated and left for drying in the air at room temperature. The dried seeds were planted in the experimental field of Biological Research Centre, University of Karachi on 31st December, 2010. The seeds germinated after 10-15 days. After three months i.e. in April 2011, the seedlings having 6-10 leaves, were transplanted in the experimental field of the Department of Zoology, University of Karachi. A total of 300 plants were transplanted in May, 2011 and were identified as pure male (XY), female (xx) and hermaphrodite (XYh) on the basis of bearing male, female and male + female (bisexual) flowers in March 2012.

The observation of pure male, female and hermaphrodite plants were first taken on 16th March, 2012. According to 1st observation a total of 185 plants were identified. Among the 185 plants, there were 80 female plants (43.24%), 82 male plants (44.32%) and 23 hermaphrodite plants (12.43%). The hermaphrodite plants are shown in Figs. 1(D-F). Then the observations were again done on 2nd May, 2012. According to this observation a total of 209 plants were identified from 300 plants. Among these 102 plants (48.80%) were identified as female (XX) plants, 81 plants (38.75%) as male (XY) plants, whereas 26 plants (12.44%) were hermaphrodite (XXY) plants.

The bisexual flowers in hermaphrodite plants are shown in Figs. 1(A-C). The study and observation was done only on hermaphrodite plants, which bore fruits. The seeds from hermaphrodite fruits were sown in May, 2012. The seedlings from these seeds were transplanted in June 2012.These plants were used for the present experiment as shown in flow chart, in the result section.

The ripe fruits from hermaphrodite plants were harvested during 16th March, 2012 to 2nd May, 2012, from time to time as shown in Figs. 1(G-I). The ripe fruits were plucked, opened (as shown Figs. 1(J-L) and the seed(s) if present were sown in pots. The pots were kept in sequence according to the date of sowing one after the other. In this way, the seedlings from male plant fruit seeds were prepared. The seedling was transplanted in the field. Again 300 plantlets/seedlings from identical male plant seeds were planted in separate rows, during the months of June, 2012.

Results

The seedlings from identical male plant, transplanted in June, 2012 cleared the picture of male and female during November, 2012, but still 50 plants (26.66%) of them yet had no male or female indication, until 19th November, 2012 (because papaya flower in 6-12 months. Only 250 (83.33%) plants were studied as male and female among which 59 were female and 191 were male plants, showing totally 23.6% female (♀) and 76.4% male (♂) plants respectively, The remaining 50 plants were also identified on 31st March 2013.

According to the identification made on 31st March 2013 100% plants were identified on the basis of flower bearing among 300 plants, 20 plants (6.67%) were hermaphrodite (XYh = XXY) as shown in Fig. 1 (M), whereas 110 plants (36.66%) were female (XX) as shown in Fig. 1(N), while 170 (56.66%) were male (XY) as shown in Fig. 1(O). The results are shown in the Flowchart and Table 1.

Fig. 1. (A), Female flower with mature male flowers in male plant; (B), Large size female flower with fruit in male plant and un-opened male flowers; (C), female flower with fruit and many un-matured male flowers; (D), many unripe (green) elongated fruits in a male plant; (E), many unripe (green) fruits in another male plant; (F), many unripe (green) fruit in a male plant of oval or round shape, as usually found in female plants; (G), showing an elongated ripe fruit from one male plant and the other pear shape from the other male plant, (H), one unripe (green) fruit and the other ripe fruit showing together in a male plant; (I), ripe fruit with green skin from upper side and eaten by birds to show seeds; (J), a ripen (yellow) fruit showing the seeds in it and the long fruit stalk (usually found in male plant fruit), but is not found in the female plant fruit. (K), a ripe fruit of another identical male plant showing the seeds in it; Fig. (L), a ripe fruit from another male plant showing, the long stalk and seeds in it. This is the same fruit which is shown in fig. 9 in the attached condition to the plant. (M), an identical male (hermaphrodite) plant, grown, from the seeds of an identical male (hermaphrodite) plant. (N), a pure female plant grown from the seeds of hermaphrodite plant. (O), a pure male plant grown from the seeds of hermaphrodite plant.

Table 1. Showing, the observations for male, female and hermaphrodite plants with percentage in bracket, during the field experiment.

S. #	Observation date	Total transplanted plants	T. plant identified	Male (XY)	Female (XX)	Hermaphrodite (XXY)
01.	16th March, 2012	300 plants from female fruit seeds	185 (61.66%)	82 (44.32%)	80 (43.24%)	23 (12.43%)
02.	2nd May, 2012	300 plants from female fruit seeds	209 (69.67%)	81 (38.75%)	102 (48.80%)	26 (12.44%)
03.	19th November, 2012	300 plants from identical male fruit seeds	250 (83.33%)	191 (76.4%)	59 (23.6%)	Expected to appear in March-June 2013
04	31st March 2013	300 plants from identical male fruit seeds	300 (100%)	170 (56.66%)	110 (36.67%)	20 (6.67%)

Hermaphrodite (XYh = XXY)

Male plants (XY) ↑ female plants (XX)

↖↗

Transplantation of seedlings from identical male plant fruit seeds

↑

Seedlings from seeds of identical male plant

↑

Germination of seeds from identical male plant fruit

↑

Sowing of seeds from identical male plant fruit

↑

Collection of seeds from identical male plant fruit

↑

Ripe (yellow) Matured fruit from identical male plant

↑

Separate male (XY) + female (XX) flowers on the same plant

↑

Identical male (hermaphrodite) plant of *Carica papaya* (XXY = XYh)

Flowchart showing the steps (procedure/sequence) of origin and evolution of female (XX) plant from an identical male (hermaphrodite) (XXY) plant seeds.

Discussion

The genus *Carica* has 35 unisexual species, of which 32 species are dioecious and 3 species are monoecious (Nicolas *et al*., 2005). However, Storey (1976) reported different type of plants (polygamous) in papaya i.e., male, female and hermaphrodite.

Liu *et al*., (2004) reported that pure male (XY) and hermaphrodite (XYh = XXY) are evolved from a single source because they are nearly identical to each other. On the other hand in the present work it has been concluded that hermaphrodite (XXY = XYh) contains the ability to give rise not only to pure male (XY) but also it give rise to pure female plants (XX), which clearly means that, the evolution of pure male (XY) and pure female (XX) has taken place from a single source of hermaphrodite (XXY) plants. As the hermaphrodite plants have XXY, therefore the sex - chromosome for male (XY) and female (XX) both are present in the same source. It has already been stated that pure male (XY) and hermaphrodite (XXY) are nearly identical to each other as per the report of Liu *et al*., (2004) but a female can be evolved due to non-disjunction, with or without fertilization as has been proved in the present work. No any hormone or chemical was given to the experimental plants in the field.

Non-disjunction during meiosis is a well understood, accepted and also well reported both in plants and animals, in which during meiosis (Anaphase 1) homologous chromosome fail to separate from each other. As a result one gamete gets a double chromosome namely XX or XY, whereas the other gets no chromosome i.e., zero-chromosome in the case of normal male and normal female, when these gamete are fertilized, one of them will get 3 chromosomes i.e., XXX, XXY or XYY and the others who had zero-chromosome, will have only one chromosome i.e., 0X or 0Y. Here all survive except YY, which is a case of lethal male, because there is no survival gene which is present on X-chromosome only. We get the following results as shown in Table 2.

Table 2. Possibilities of Non-disjunction having XX or XY double chromosome in gamete of hermaphrodite and fertilization with normal female gamete X, X and male gamete X, Y.

1	XXY	↗→	XX fertilization with gamete + X = XXX	Super female	Survive
		↘→	Y fertilization with gamete + Y = YY	Lethal male	Not survive
or	XXY	↗→	XX fertilization with gamete + Y = XXY	Hermaphrodite	Survive
		↘→	Y fertilization with gamete +X = XY	Pure male	Survive
2	XXY	↗→	XY fertilization with gamete + X = XXY	Hermaphrodite	Survive
		↘→	X fertilization with gamete + Y = XY	Pure male	Survive
or	XXY	↗→	XY fertilization with gamete + Y = XYY	Super male	Survive
		↘→	X fertilization with gamete + X = XX	Pure female	survive

The important point to note in this field experiment was the production of bisexual flowers in a dioecious plant. In this bisexual flower androecium was fused with the ovary. It may be due to the dual nature of the central X-chromosome, contributing genetic material to both male and female sex or over lapping of the X-chromosome for both male and female plants. Possible contribution of genetic material to both male and female plants by the central X-chromosome of an identical male is shown as follows.

Dual nature of central X-chromosome to both male and female.

↔	→	→
X**X**Y⇨	X**XX**Y⇨	X**X** + **X**Y
↔	←	←

This condition was also noted in hermaphrodite XXY plant, during the field experiments, that the XXY-chromosomes during genetic gamete formation may get separated as X, X, Y and after self or cross fertilization, with normal XX, XY or XXY, they may result in pure male or pure female sex plants, as shown below.

Self or cross fertilization of hermaphrodite by XY (male).

	X	**X**	**Y**			**X**	**Y**
X	XX	XX	XY	OR	**X**	XX	XY
Y	XY	XY	YY		**X**	XX	XY
					Y	XY	YY

Female (XX) 2: Male (XY) 3, Lethal (YY) 1 (same result in both cases).

Self or cross fertilization of hermaphrodite by XX (female).

	X	**X**	**Y**			**X**	**X**
X	XX	XX	XY	OR	**X**	XX	XX
X	XX	XX	XY		**X**	XX	XX
					Y	XY	XY

Female (XX) 4: Male (XY) 2 (same result in both cases).

Cross fertilization in between hermaphrodite and hermaphrodite.

	X	**X**	**Y**
X	XX	XX	XY
X	XX	XX	XY
Y	XY	XY	YY

Female (XX) 4: Male (XY) 4 (YY) is the lethal case, not survive).

Now, if there is a case of non-disjunction then the hermaphrodite will also appear as stated before in Table 2 (above), which is also in line with the findings of Liu *et al*., (2004), who reported that a hermaphrodite plant and a pure male are nearly identical to each other because they have evolved from a single source. Therefore in the present findings as well, not only the pure male and the hermaphrodite are evolved from a single source of XXY-chromosome but also the pure female (XX) also evolved from the same single source.

Fujisawa *et al*., (2001) reported the origin of female *Marchantia polymorpha* (liverwort) DNA fragment (markers) chromosome from male XY-chromosome, in ratios of 2 female to 6 male DNA fragment, a ratio of 1 female: 3 male. The present study is also in line and support of the findings of Fujisawa *et al*., (2001), because in the present work 24% female and 76% male were recorded, which means that the ratio of male and female was nearly, 1 female: 3 male, (Table 1, Observ. #3) which is similar to the findings of Fujisawa *et al*., (2001).

Separate sexes have been evolved repeatedly from hermaphroditic ancestors in flowering plants and thus select taxa can provide unparalleled insight into the evolutionary dynamics of sex chromosomes that are thought to be shared by plants and animals alike Goldberg *et al*., (2010). In the present work a pure male and a pure female has been recorded practically in this field work.

Wu *et al*., (2012) reported the propagation of auxiliary buds and terminal buds from hermaphrodite plant by tissue culture for mass production of papaya fruit, but in the present work the natural method of propagation (NMP) was adopted in the field level for the study of origin and evolution of pure female plants from the seeds of hermaphrodite plants.

Previously the scientists used mm for female, mM for male and mMh for hermaphrodite, but the humans have X / Y- chromosome system. Therefore, if, m = X, M = Y, and Mh = Yh = XY then: mm = XX (female), mM = XY (male) and mMh = XYh = XXY (hermaphrodite). "Papaya is a major fruit crop in tropical and subtropical regions worldwide. It is trioecious with three sex forms: male, female, and hermaphrodite. Sex determination is controlled by a pair of nascent sex chromosome with two slightly different Y-chromosome, Y for male and Yh for hermaphrodite. The sex chromosome genotypes are XY (male), XYh =XXY (hermaphrodite), and XX (female)" as per report of Hofmeyr (1938, 1939, 1967), Storey, (1938, 1953, 1976), Na, *et al*., (2012).

The most important point noted above is that the single hermaphrodite (XYh = XXY) plant gave rise to all three sexes (male, female and hermaphrodite) from a single plant source, in field level experimental study of three years (2011-2013), whereas Gschwend *et al*., (2011) reported "papaya is trioecious with three sex types: male, female and hermaphrodite. In any given breeding system of papaya, it is either dioecious with male and female or gynodioecious with hermaphrodite (means Yh) and female (means XX). No papaya plant produces all three sex type due to the lethal effect of any combination of the Y and Yh chromosomes".

Now according to Gschwend *et al*., (2011) papaya plant is trioecious, here hermaphrodite (Yh) and female (XX) i.e., Gynodioecious = Yh + XX or simply XXY. Therefore the karyotype of an identical male (hermaphrodite) Gynodioecious may be written as 2N + 1 = 18 + 1 = 19 or 16 + 3 = 19 or simply 16 + XXY = 19, where as the karyotype for pure male papaya may be given as 2N =18 or 16 + 2 = 18 or simply 16 + XY = 18, while for pure female papaya plant, the karyotype may be given as 2N = 18 or 16 + 2 = 18 or simply 16 + XX = 18, as Na *et al.* (2012) reported 60 + XXY = 63 for male Echidna (a primitive mammal), Rebagliati *et al*. (2005), reported 12 + XXY = 15 for stink bug (Pentatomid) and Tariq (2011a) reported 44 + XXY = 47 chromosome in human male baby.

The identical male plant (XXY) was found to be fertile, because it produced normal pure male (XY) and normal pure female (XX) in the present findings. As the papaya chromosomes have virtually all of the features (characters) same as the human chromosomes Liu *et al*., (2004), therefore the above experimental finding may be applied on humans as well. In humans even now, in this modern world, male (one out of 1000) babies are getting birth with XXY-chromosome because of the reason that this condition of chromosome was inherited from the 1st male human on earth, the Aadam, who had the XXY-chromosome condition i.e., 2N + 1 = 46 + 1 = 47 or 44 + XXY = 47 as reported by Tariq (2011b). The identical male (hermaphrodite) is usually found with one extra X-chromosome. This research also supports the theory that the plants and the animals have originated from hermaphroditic ancestors.

Conclusions

In all cases a hermaphrodite individual cross fertilized by another pure male (XY) or hermaphrodite fertilized by the pure female flower on the other plant or the hermaphroditic flower is self fertilized by its own androecium fused with the gynoecium, or a bisexual flower is crossed by another bisexual flower. Female sex evolution took place in all these cases. Therefore a hermaphrodite individual gave rise not only to pure male and hermaphrodite but also to a pure female sex, as reported by Fujisawa *et al*., (2001). This experiment also proves that an identical male having XXY-chromosome gave rise to pure normal male and pure normal female, because, the hermaphrodite (mMh = XYh = XXY) is fertile.

This may also be accepted for human being as well, as the papaya has virtually the same characters in its sex chromosomes, as humans have, as per the report of Liu *et al*., (2004) and that not only the pure male (XY) and an identical male (mMh = XYh = XXY) have evolved from a single source but also the pure female (XX) as well. This is the 1st report on propagation of three sex in papaya from the seeds of hermaphrodite plant identical to male plant having mMh= XYh = XXY-chromosomes.

Before this report all reports are from clonal, terminal, lateral, axillary buds and tissue culture for the propagation of papaya but not from the seeds of hermaphrodite plants. This is also proved in this research that the dioecious flowering plants have evolved independently from hermaphroditic ancestors as reported by Yampolsky & Yampolsky (1922); Renner & Ricklefs (1955); Takhtajan (1969); Goldberg *et al*., (2010), Tariq (2011ab) and Tariq (2013).

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