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Papaya Breeding; Recent Advances and Future Prospects

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Papaya Breeding; Recent Advances and Future Prospects[1]

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ABSTRACT

Papaya [Carica papaya L.] is one of the most popular and nutritious tropical fruit in the world, and it is gaining popularity among the growers day by day. Papaya breeding strategy such as popular germplasm of papaya, hybridization and heterosis, mutation breeding, breeding steps followed in Malaysia, molecular markers for sex determination in papaya, disease resistance breeding in papaya is reviewed. Papaya research achievement in breeding, molecular breeding in India, Malaysia, China, Brazil,Taiwan, Bangladesh are considered. Breeding challenges like nutritional quality development, sex determination in gene level, disease resistance, molecular diversity analysis is reviewed well. Future prospects of papaya research mainly emphases on papaya biochemical and molecular genetics and breeding, deepening the functional genome research to reveal special gene resources, enhancing the molecular genetic research for breeding. Conducting related species gene study to enhance disease resistant germplasm innovation, improving gene transformation technique to use exogenous genes, perfecting in vitro protocol should be integrated in the future papaya research in order to cope up with the currently existing ecological phenomena.

Keyword: Papaya breeding, molecular markers, achievements, scopes.

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CHAPTER 1

INTRODUCTION

Papaya (*Carica papaya* L.) is one of the world's top 10 tropical fruits, belonging to the small family *Caricaceae*, which consists of 6 genera and 35 species (Ming *et al*., 2005). Over 0.35 billion years ago the *Caricaceae* family was spread to Central America though it was derived from Africa (Carvalho & Renner, 2014). *Caricaceae*moved from Central America to South America between 0.27 and 0.19 billion years ago (Carvalho & Renner, 2014). Despite the controversy surrounding the details of its origin, sufficient information has been gathered (Chávez-Pesqueira&Núnez-Farfán, 2016) to show that papaya originated from the Caribbean islands of Mesoamerica.

Nowadays it is becoming an important international fruit, both as fresh fruit and for processed products. It is third most cultivated fruit in the world which occupies 15.36% of total tropical fruit production in the world (Paltali&kumar, 2016). The total world production of papaya in 2018 was 13.3 million of tones (Food and Agriculture Organization Corporate Statistical Database [FAOSTAT], 2018). In Bangladesh papaya is getting popularity day by day because of its highly nutritious properties. . Christopher Columbus had called papaya 'the fruit of the angels' because of its aroma, sweetness and cream like consistency what makes it so popular. Bangladesh founds good quality of papaya crop mainly from greater Rajshahi, Pabna and Jessore (Chowdhury & Hasan, 2013). According to the report of BBS (2019), in the year of 2018-19 papaya is cultivated in 38350 acres of land giving production of 135809 metric tons. Although cultivation area has become doubled production has not increased so much. A more reliable technology of sex type determination, disease and pests, lack of good cultivars hinders the production of papaya. Till today in Bangladesh there are two released varieties of papaya available for cultivation by the enthusiastic farmers. These two varieties are BARI Papaya-1 and BU-Papaya -1. BARI Papaya-1 (Shahi papaya) is an excellent variety, but its fruit quality has been degenerated due to lack of maintenance breeding (Ara *et al*., 2016). And Bu-Papaya is released in 2012 from the Dept. of Genetics and Plant Breeding of Bangabandhu Sheikh Mujibur Rahman Agricultural University. It is high yielder, gynoedioecious, produce 98% female plant. But for further research it is vital to know the past achievement and present research on papaya to improve its production in Bangladesh. Along with this it is also important to know the main extent of papaya to be work upon in future. In this paper we will see how priority is being changed in papaya breeding.

The scientists concentrated on quality development of papaya from heterosis breeding. Backcross breeding with microsatellite marker association creates a need to research in finding molecular diversity and progress is needed in transgenic methods. Papaya being the first genome sequenced crop (Wei & Wing, 2008) has opportunity to render further research in manipulation of sex determining genes from present molecular marker assisted breeding. Emergence of new viral diseases is stimulating the geneticists to search for resistant gene in related species of papaya. And thus this review paper presents a comprehensive knowledge of the success and scope of present research and future research area on papaya.

Objectives:

- To know the breeding advances of papaya (past and present)
- To review future papaya breeding scopes

CHAPTER 2

MATERIALS AND METHODS

This seminar paper is exclusively a review paper. So, no specific methods of studies are followed to prepare this paper. All facts and data were composed and recycled from secondary sources. This seminar paper has been prepared by reciting different books, journals, booklets, proceeding, newsletters, consultancy report which are available in the online libraries of BSMRAU & internet. Some information is collected from BARI and some private and international agricultural organizations. Maximum necessary supports were taken from internet searching. Finally, this seminar paper was prepared with the consultation of my respective major professor and honorable seminar course instructors.

CHAPTER 3

REVIEW OF FINDINGS

Carica papaya is an adherent of the *Caricaceae* family and is the most frugally vital species in the family (Carvalho & Renner, 2012). India is the highest producer of papaya, Fig.1 represents the worldwide production of papaya in 2018shared by different countries (FAOSTAT, 2019) C. *papaya* is the only member of the genus which is being restored from the *Vasconcella*considering a part of the genus *Carica*, until the year 2000 (Badillo, 2000). It is a cross pollinated species with mainly three sex types: female, male, and hermaphrodite (Fig 2). Most individuals are dioecious in wild population, however cultured papayas are dioecious or hermaphrodites (Carvalho & Renner, 2012; Chávez-Pesqueira*et al*., 2014).Sex determination for the three types of papaya, female, male, and hermaphrodite plants, is genetically controlled by the pairing of sex chromosomes (Carvalho & Renner, 2012), over a sex-linked region that acts like an XY sex chromosome. For male and hermaphrodite entities, sex is controlled by somewhat dissimilar Y chromosome regions: Y_h in hermaphrodites and Y in males (VanBuren*et al*., 2015). Female plants yield flowers and fruits all year round in tropical regions; though, in subtropical areas, even with continuous flowering, fruit set is reduced in drier seasons (Gonsalves, 1998).

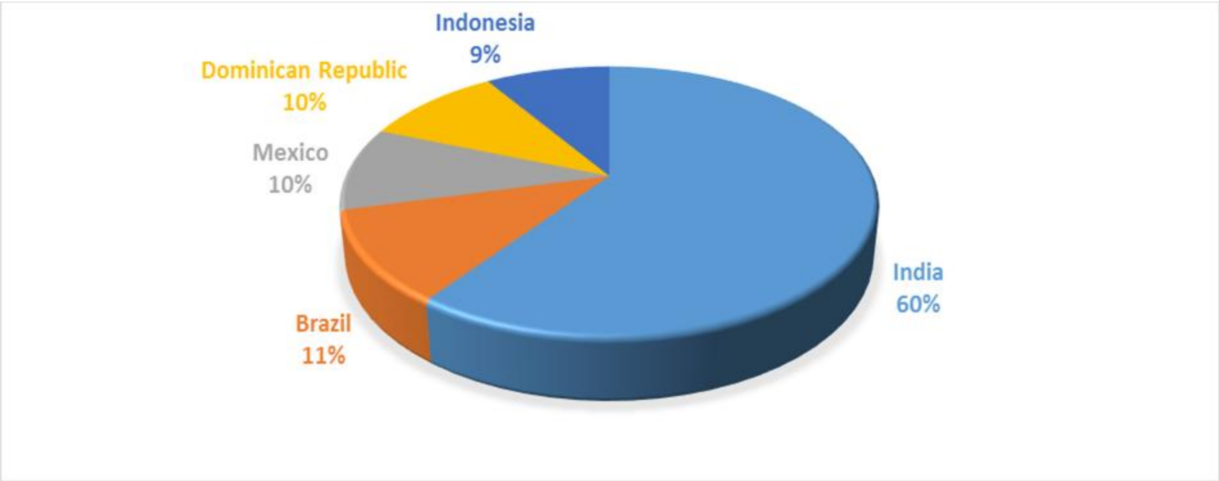

Fig 1: Worldwide papaya production in 2018.

(Source: FAOSTAT 2019)

3.2. Recent estimates of papaya production in Bangladesh:

Papaya farming gains popularity in Bangladesh. Cultivation of papaya has gained immense

a. b. c.

Fig 2: Papaya flowers (a. Female, b. Male, c. Hermaphrodite).

(Source: Anh *et al*. 2011)

popularity among the farmers of several district from the last few years. It has important sources of income generation leading to economic self-reliance in the area. The Fig 3 shows the production of papaya in different division of Bangladesh and reveals that Rajshahi and Chattagram gives the highest production in 2018-19. Dhaka and Khulna also gives a large scale of production and in all eight division cultivation of papaya is occurred.

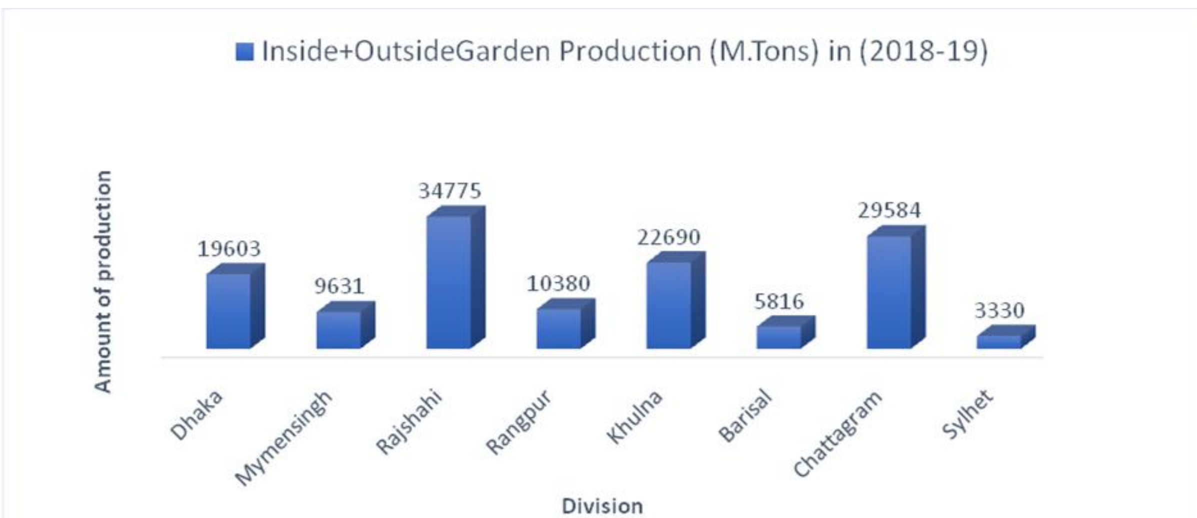

Fig 3: Production of papaya in different division of Bangladesh in 2018-19.

(Source: BBS, 2019)

3.3. Popular germplasms of papaya in the world

There are many varieties of papaya cultivated in the world. Table 1 represents some popular varieties of papaya (with their sex type) in the world.

Table 1:Different types of papaya varieties cultivated around the world

Gynodioecious varieties	Dioecious varieties
Guinea Gold	Improved Petersen
Kapoho Solo	Sunnybank/S7
Sunrise	Richter/Arline
Waimanalo	Homestead
Rainbow	Betty
Maradol	Cariflora
Cartagena	Coimbitor
CoorgHoneyDew	Hortus Gold
Semangka	Honey Gold
Dampit	
Eksotika	
Sekaki	
Cavite	
Tainung No. 5	
Sai-nampueng	
Khaek Dum	

(Source: International Tropical Fruits Network [TFNet], 2016)

3.4. Nutritional quality of papaya

Fresh papaya fruit (100 g) supplies 179 kJ of energy, 11 g carbohydrate, 1.7 g fiber, and less than 1 g protein or fat (United States Department of Agriculture [USDA], 2009). Ripe papaya has a significant amounts of folate and minerals (Hardisson*et al*., 2001; Wall 2006; Saxholt*et al*., 2008; USDA 2009). Papaya's most important nutritional benefit comes from vitamin C (ascorbic acid), followed by the pro vitamin A carotenoids (β-carotene and β-cryptoxanthin). Ascorbic acid is a potent, water-soluble antioxidant present in ripe papayas. Papaya ranked second among 44 fresh fruits and vegetables for vitamin C content, exceeding oranges, grapefruit, and broccoli

(Franke *et al*., 2004). Table 2 represents the nutrients content of papaya according to USDA, 2009 report.

Table 2: Nutritional values for papaya fruit (per 100 g edible fresh weight)

Nutrients	Values	Nutrients	Value
Water(g)	88.1	Fructose(g)	3.73
Energy(kJ)	179	Vit C (mg)	60.9
Protein(g)	0.47	Vit A (micro g RAE)	47
Fat(g)	0.26	Folate (micro g)	37
Carbohydrate(g)	10.82	Thiamin (mg)	0.023
Dietery Fiber(g)	1.7	Riboflavin (mg)	0.027
Phosphorus (mg)	10	Niacin (mg)	0.357
Glucose(g)	4.09	Pantothenic acid (mg)	0.191
Vit B6 (mg)	0.038	Magnesium(mg)	21
Calcium (mg)	20	Potassium (mg)	182
Iron (mg)	0.25	Sodium (mg)	8
Zinc(mg)	0.08	Copper(mg)	0.05
Manganese (mg)	0.04	Sucrose (g)	7.82

(Source: USDA, 2009)

3.4. The Achievements of papaya genetic improvement and breeding

There are several researches have been done in papaya breeding and some research need to progress more, we will learn some important research among them.

3.4.1 Stepwise priorities in papaya breeding practiced in MARDI

Many achievements have been gained in papaya breeding still now.Systematic breeding for improved papaya varieties in Malaysia was started 36 years ago in Malaysian Agricultural Research and Development Institute (MARDI) (Chan, 2007).At the early stage, importance was given on selection of ‘baseline cultivars’ from growers’ fields. An addition breeding program puts prominence on eating quality by backcross breeding with ‘Sunrise Solo’ as the recurrent parent in improving the eating quality of 'Subang 6' results in release of the 'Eksotika'.To improve the fruit cosmetics they release 'Eksotika II' which has more attractive, firmer flesh (Chanw, 2005).In wide crosses, heterosis in yield of hybrids was an astounding 199.6% over the better parent. The results from the hybrid program of MARDI reveals that F1 hybrid varieties are far superior to self-pollinating pureline varieties.The outbreak of papaya ringspot virus disease in 1991 also saw a shift in emphasis to develop disease resistance lines i.e. L41, L90, L248 (Chan, 2004a, 2005).Improving the shelf life of 'Eksotika' papaya with delayed ripening characteristics results in 'Eksotika 3'. Future priorities may focus on breeding for 'designer' papayas with improved nutritive values in vitamins and antioxidants along with novelty in serving the fruit with negligible management (Chan, 2005).The Table 3 represents the stepwise priorities in papaya breeding of MARDI according to Chan (2005).

Table 3: Stepwise priority and achievement in papaya breeding in MARDI (1970-2015)

Area of Improvement	Year	Variety realeased
Uniformity	1970 to 1975	Subang 6 (Chan &Ooi, 1975)
Fruit Quality	1972 to 1987	Eksotika 1 (Chan, 1987)
Cosmetics	1989 to 1993	Eksotika 2 (Chan, 1993)
Yield and Stability	1989 to 2001	Sekaki x L19 (Chan, 2001)
Disease resistance	1991 to 2015 and further	L19, L90, L248 (Chan & Ong, 2003)
Fruit storage life increase	1996 to 2015 and further	Eksotika 3 (Chan, 2005)
Designer papaya	2005 to 2015 and further	Yet to develop (Chan, 2005)

(Source: Chan, 2005)

3.4.2. Hybridization, heterosis and mutation breeding in papaya

Intervarital and intergeneric hybridization was practiced in papaya. But still there is great scope for development of superior cultivars with better quality and yield. At Tamil Nadu Agricultural University (TNAU), three varieties have been developed viz. CO3 (CO2 x Sunrise Solo), CO4

(CO1 x Washington) and CO7 (CP.75 x Coorg Honey Dew). AtThe Indian Institute of Horticultural Research (IIHR) Bangalore, two hybrids IIHR-39 named as Surya (Sun Rise Solo x Pink Flesh Sweet), and IIHR-54 (Waimanalo x Pink Flesh Sweet) were developed (Dinesh & Yadav, 1998), Hybrid HPSC-3 (Tripura local x Honey dew) was developed by the Indian Council of Agricultural Research (ICAR) Tripura (Singh & Sharma, 1996). Cultivar Cariflora a promising cultivar tolerant to PRSV, was developed by crossing K_2xK_3 line of papaya (Conover *et al*., 1986). Dai (1960) reported heterosis in the cross between Philippines x Solo varieties. F1 hybrid have a tendency to give less number of seed and improved plant vigour. Heterosis up to 111.4% for yield and yield traits was obtained in Solo yellow x Washington whereas high heterosis for potential economic competitiveness was noticed in Thailand x Washington (Iyer&Subramanyam, 1981). At IIHR, Bangalore, an F1 hybrid namely, Surya (Sun Rise Solo x Pink Flesh sweet) was released which was gynodioecious. By mutation breeding Ram & Majumder (1981) developed a dwarf mutant line by treating papaya seed with 15K gamma rays. At first, 3 dwarf plants were isolated from M2 population. Repeated sibmating among the dwarf plants helped in establishing a homozygous dwarf line PusaNanh about 600-800g, the flesh is red in colour, firm, sweet taste with a TSS of 14º brix (Mitra & Dinesh, 2017).

3.4.3. A Recent breeding scheme of papaya

Drew (2016) stated to improve plant through conventional breeding, marker assisted breeding and genetic modification. Moreover, they developed segregation populations for future breeding work which will facilitate future research and increase selection of new commercial varieties. They followed the following strategy in improving papaya breeding.

1) Plant and grow several lines of Papaya to maturity
2) Identified important trait for papaya breeding program
3) Evaluated traits of interest in all the lines
4) On first harvest the collected data, selected trees bearing fruit having best consuming quality, cross pollinated the selected trees with commercial varieties with a view to get segregating populations with improved eating quality
5) On second harvest they collected data on correlation and studied the repeatability
6) On third harvest they select two tress for each trait with highest difference and finally cross

Pollinate those trees to establish segregating populations for marker analyses.

3.4.4. Molecular markers in sex determination of papaya

Hofmeyr (1938) and Storey (1938) independently proposed the hypothesis that sex determination in papaya is controlled by a single dominant gene with three alleles. They anticipated the genotype of male, hermaphrodite, and female plants are Mm, Mh_m, and mm, respectively, and described that homozygous dominant alleles are lethal.Therefore, segregation of sex type from selfed hermaphrodite trees is 2 hermaphrodites: 1 female.Whereas seeds from female trees segregate at the ratio of 1:1 female: hermaphrodite if the plant is crossed with a hermaphrodite tree, but that of 1:1 female: male when it is crossed with a male tree (C. Kanchana-udomkan*et al*., 2014). Molecular markers are authentic tools and have been used to simplify genetic improvement in various crop species including *Carica papaya* (Eustice*et al*., 2008).The first report of a sex-linked marker in papaya was reported by Sondur*et al*. (1996). The first PCR-based markers for papaya were randomly amplified polymorphic DNA (RAPD) applied to determine sex of papaya prior to flowering (Aryal& Ming, 2014; Stiles *et al*., 1993). FurtherRAPD and DNA amplification fingerprinting (DAF) were compared revealed that DAF reactions were more reliable (Somsri*et al*., 1998).From 2000 ahead, the sequence-characterized amplified region (SCAR) technique has been used. The following Table 3 informed us about some sex detecting markers of papaya.

3.4.5. Detection of quantitative trait loci in papaya for breeding improvement

Many agronomically important traits, i.e. fruit size, fruit shape, flesh flavour and skin quality arequantitative traits that are influenced by multiple genes. Fruit shape in papaya has long been recognized as a sex-related trait with female fruit exhibiting a rounder, spherical shape and hermaphrodites exhibiting a more elongated, pear to cylindrical shape.Various quantitative trait loci (QTLs) have been identified in many crops; however, surprisingly not many QTLs have been identified in papaya breeding research. Recently, in 2012, a QTL analysis for papaya fruit size and shape has been reported (Blas *et al*. 2012). A total of 14 significant QTL were detected by Blas *et al*., (2012) from F2 mapping population generated from a cross between a Thai cultiverKhaek Dum (female) and a mutant 2H94 (hermaphrodite, pollen donor) was grown at the Hawaii Agriculture Research Center, Kunia Substation on Oahu, Hawaii. QTL were detected for the papaya fruit traits weight, length, diameter and shape. Significant correlations were observed for each phenotypic fruit trait pair except for between diameter and length could aid breeders in specifically tailoring new varieties to consumer preferences.

Table 4: Different types of sex determining markers of papaya

Type of Marker	**Name of Marker**	**Sex detection**	**References**
RAPD	PSDM	Hermaphrodite and Male	Urasaki*et al*. (2002b)
RAPD	BC210 438	Hermaphrodite	Lemos*et al*. (2002)
RAPD	OP-Y7 900	Male	Chaves-Bedoya and Nuñez (2007)
RAPD	OPF2-0.8	Male	Parasnis*et al*. (2000)
SCAR	T1	All sex	Deputy *et al*. (2002)
SCAR	T12	Hermaphrodite and Male	Deputy *et al*. (2002)
SCAR	W11	Hermaphrodite and Male	Deputy *et al*. (2002)
SCAR	SCARps	Hermaphrodite and Male	Urasaki*et al*. (2002b)
SCAR	SCARpm	Hermaphrodite and Male	Urasaki*et al*. (2002b)
SCAR	SCAR	Hermaphrodite and Male	Chaves-Bedoya and Nuñez (2007)
SCAR	C09/20	Hermaphrodite and Male	Niroshini*et al*. (2008)
SCAR	OPF2-0.8	Male	Parasnis*et al*. (2000)
DAF	OPA06	Hermaphrodite and	Somsri and

		Male	Bussabakornkul (2008)
RFLP	(GATA) 4	Hermaphrodite and Male	Parasnis*et al*. (2000)

(Source: C. Kanchana-udomkan *et al*., 2014)

3.4.6. Disease resistance breeding in papaya

PRSV-P is the greatest problem for papaya production worldwide and it is a great threat for the producers.For the papaya ring spot virus, however, early evidence of quantitative inheritance of tolerance to ring spot virus in papaya was obtained from the selection of individual tolerant lines of papaya. In crossing experiments, these lines showed that the tolerance was greatest when both pistillate and staminate tolerant lines were used (Conover &Litz, 1978). Many researchers took interspecific hybridization step to cross C. *papaya* with PRSV-P resistant related *carica*species (C. *cauliflora,* C. *pubescens,* PhytophthoraresistanceC. *goudotiana*etc.). A vastly effective procedure was developed to rescue and germinate C. *papaya* xC. *cauliflora* immature embryos (Magdalita, *et al*., 1996) and later on the procedure has been adapted to produce hybrids between C. *papaya* and PRSV-P resistant species C. *quercifolia* and C. *pubescens*. And those hybrid plants had grown robustly in the field, and a few C. *papaya* x C. *quercifolia* plants have produced some viable pollen. (Drew *et. al*., 1997) Later on with the advancement of breeding tools a variation of RAPD technique, which employs increased annealing temperature and polyacrylamide gel detection. Randomly amplified DNA fingerprinting (RAF), identified markers linked to papaya ringspot virus type P (PRSV-P) resistance in the related *Vasconcelleapubescens* (Dillon *et al*., 2005).

3.4.7. Biotechnology

Papaya transgenic cultivars Rainbow and Sunup resistant to PRSV were successfully developed and used for commercial cultivation in Hawaii (Gonsalves, 1998, 2006). Papaya breeding lines have been developed, even if with limited success, with transgenic resistance to papaya ring spot virus (Davis & Ying 2004).

3.5. Future prospects

3.5.1. Crop nutritional quality development

The primary sources of variation for nutritional plant products are genetic, pre harvest environment, maturity at harvest, and postharvest conditions. Thus at this time, the best prospects for enhancing papaya nutritional content are to maximize the genetic potential within the existing papaya germplasms through optimal varietal selection, orchard edaphic factors, crop management, harvest maturity, and postharvest treatments and storage temperatures. Nevertheless, major advances in papaya genetic tools provide an avenue for further germplasm improvement. Combining classical breeding approaches with marker-assisted selection appears to be a practical and efficient strategy for papayas. A backcross program linked with microsatellite marker selection was demonstrated for introgression of desirable genes into an elite recurrent parent (Ramos *et al*., 2011). A comprehensive high-density genetic linkage map for papaya is available and useful for mapping quantitative trait loci and for marker-assisted selection (Blas *et al*., 2010).The availability of this genomic information enables future in-depth studies directed at the nutritional aspect of papaya fruit. Differentially expressed gene transcripts associated with fruit ripening have been described, including several related to vitamin C, biotin and folate biosynthesis, and precursors for carotenoids (Fabi*et al*., 2010). Many nutritionists and consumers are aware of the dietary and aesthetic value of papayas. Further increases in nutritional content are possible if the germplasm base can be diversified, either through traditional or transgenic methods, including through inter secondary compounds and attempts to regulate key genes in biosynthetic pathways could lead to unintended consequences of lower yield, aroma, flavor, or visual quality.

3.5.2. Improvement of disease resistant breeding

Many disease resistances are found in wild relative *Vasconcellea* species and future breeding may require interspecific recombination to reintrogress these back into the elite cultivated genomes (Coppens *et al*., 2013).In summarizing their genetic diversity, it should be noted that the *Vasconcellea*species have recently been divided into three clades (Coppens *et al*. 2013). They are (1) V. *weberbaueri*, V. *stipulata*, V. *xheilbornii*, and V. *parviflora*; (2) V. *chilensis*, V. *candicans*, V. *quercifolia*, and V. *glandulosa* ; and (3) a clade holding all other species of the genus (Coppens *et al*., 2013). By advancing techniques additional wild relative traits may be introgressed into papaya in the future. A survey was conducted from 2006 to 2008 at the plantations of papaya at diverse sites in India, discovered that maximum leaf curl disease occurrence (23.50%) was recorded in Lucknow district. In cultivar Co-4 80.00 and 86.67% and in MF-1 80.00 and 83.33%. However minimum leaf curl occurrence (23.33 and 20.00 percent) was detected on Harichaap cultivar during 2006-07 and 2007-08, correspondingly. Only a variety found resistant against leaf curl disease i.e. Harichaap through both the years. Moreover, CO-2, CO-3, CO-6, Coorghoneydew, Pusa Delicious and Pusa Dwarf these six varieties were found to be moderately resistant. (Singh & Awasthi, 2017). The details of their findings is in the following Table 5.

Table 5: According to Singh and Awasthi (2007),response of papaya varieties against papaya leaf curl diseases under field conditions during 2006-07 and 2007-08

Category	Grade	Disease incidence	Varieties in 2006-07	Varieties in 2007-08
Protected	1	0.00	Nil	Nil
Resistant	2	0.1 – 25.0	Harichaap	Harichaap
Discreetly resistant	3	25.1 – 50.0	CO-2, CO-3, CO-6, Coorghoneydew, Pusa,Delicious and Pusa Dwarf	CO-2, CO-3, CO-6Coorghoneydew, Pusa, Delicious and Pusa Dwarf
Susceptible To disease	4	50.1 – 75.0	CO-1,CO-5,Pusa Nanha, Pusa Mejasty,PusaGaint and Washington,	CO-1,CO-5,Pusa Nanha, PusaMejasty, PusaGaint and Washington,
Extremely susceptible	5	75.1– 100.00	CO-4, CO-7, MF-1	CO-4, CO-7, MF-1

(Source: Singh &Awasthi, 2017)

Moreover, in a recent study by Varun *et al*., (2017) vector control is discouraged for environmental hazard due to the excessive use of insecticides. They also suggested Pathogen-derived resistance by utilizing cross protection of host plants (pre-infection with milder virus strains) has limited success and high development costs for sustainable crop protection.Natural resistance in host plants can resist the viruses either directly through specific mechanisms or indirectly through resisting infestation by the insect vectors of the viruses. The varietal differences in any crop plant reflected as resistance and/or tolerance to the viruses may be attributed to this. It is thus possible to develop conventional breeding and selection approach to evolve crop plants with natural resistance to viruses. Though analysis of genetic diversity among papaya cultivars in India has been done (Saxena *et al*., 2005).In general, the success with conventional breeding methods to develop and exploit within germplasm diversity for resistance to a virus is a challenging task.Efforts are ongoing to develop transgenic papaya with respect to resistance against PaLCuV using coat protein gene and both sense and antisense replicase gene in India (Mishra *et al*., 2007). Recently,Zaidi *et al*., (2016) exploited CRISPER/Cas9 technology for single and multiple virus resistance in plants. CRISPR/Cas9 is being used to develop virus-resistant plants in two ways: either viral genome itself can be targeted and cleaved or the host plant genome can be engineered to make the crop tolerant to viruses. Complete genome of papaya has already been decoded (Ming *et al*., 2008, 2012) which makes papaya plant an ideal candidate for the use of CRISPR technology to develop resistance against viruses infecting it.

3.5.3. Development of a new in vitro protocol

Tissue culture for generating virus-free plants through in vitro techniques of plant propagation has been a choice among plant virologists. Using in vitro propagation Meena *et al*. (2014) have reported leaf curl virus-free chili plants. Papaya responds poorly to tissue culture and its latex makes in vitro propagation of papaya difficult; hence, no major efforts have been made to develop virus-free papaya using tissue culture techniques. It is a scope to develop an advance protocol to initiate in vitro propagation of papaya to get plantlets with vigour.

3.5.4. Development of a more static protocol to get a true to type hermaphrodite variety

Specific primers are now available to distinguish one flower (sex) type from the other in the pre-flowering stages (Gangopadhyay*et al*., 2007). However, from practical point of view, such markers are not useful for the farmers due to their high cost. Hermaphrodite fruit produces seeds that segregate in the ratio of 2:1 (hermaphrodite: female). Therefore, true hermaphrodite plants are nonexistent. It is now clear that papaya has a pair of nascent sex chromosomes and the two sex determination genes are in the male-specific region of the Y chromosome (MSY) (Wang *et al*., 2012; Gschwend*et al*., 2012). Therefore, manipulating sex determination gene will lead to the development of true-breeding hermaphrodite varieties.

3.5.5. Analysis of molecular diversity

SSR markers have been used for sex identification (Parasnis*et al*., 1999), in genetic diversity studies (Pérez *et al*., 2006 ; Ocampo Pérez *et al*., 2007 ; de Oliveira, *et al*.2008 , 2010a , b ; Eustice *et al*., 2008 **)** It is vital to analyze the genetic diversity of the samples to be studied before a breeding program can be launched. They used thirty-one genotypes of papaya which were collected from Spain, Brazil, Ecuador, China, Taiwan, India, and few localities in Bangladesh to study their molecular diversity along with their genetic relatedness by using SSR markers. They found 11% and 89% of the entire genetic diversity among and within the population respectively. Their findings in research expands the current papaya genetic resources, those can be essential source for the choice of parental lines within any hybrid breeding program. (Hasibuzzaman*et al*., 2020)

CHAPTER 4

CONCLUSION

In conclusion, like any other crops, the final objective of papaya breeding is to improve the quality and quantity of the product. Fruit nutritional quality development, manipulating the sex determination genes to find a static protocol for true-to-type hermaphrodite variety development, improvement of techniques to get disease resistant variety, search for variable germplasm by utilizing molecular diversity analysis, a standard in vitro protocol development are the papaya breeding objectives in the present stage and foreseeable future.

Papaya breeding has achieved a tremendous advancement. Advanced technologies have been made for breeding of papaya. As previously conventional breeding and some molecular techniques were the only source of improvement. But nowadays, different advanced breeding approaches has been taken to improve quality.

For future advancement different experiment should be done to make the crop more acceptable to the consumer. Major problems of this valuable tropical crop should be solved by applying advanced techniques. The advancing papaya breeding techniques, from individual selection to hybridization and advance molecular breeding, will benefit the current and future papaya breeding programs in the world.

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