CHAPTER-II

REVIEW OF LITERATURE

Production of fruits & vegetables in India during 2015-16 was about 286 million tons and ranked as the second largest producer in the world (NHB).India is the largest producer of papaya and produces 5.5 million tons of papayas each year. The fruit is mainly cultivated in Uttar Pradesh, Bihar, Assam, Andhra Pradesh, Tamil Nadu, Karnataka, Gujarat, Maharashtra, West Bengal, Orissa, Manipur and Meghalaya. Papaya (*Carica papaya* L.) is an economically important fruit crop and belongs to family Caricaceae. The ideal season for cultivation is the beginning of monsoon and continued from June to November. The soil condition for papaya is, sandy loam soil with depth upto 45 cm and also black soil without water logging (NHB). The leaves and fruit are commonly used by people for treatment and well-being. People with diseases like warts, corns, sinuses, eczema, cutaneous tubercles, blood pressure, dyspepsia, constipation are benefited by papaya (Akhil and Vijayalakshmi, 2015). It is a polygamous diploid (2n = 18) plant with a complex breeding system including dioecious and gynodioecious forms (Storey 1953). Papaya is conventionally propagated by seeds which are often dormant or desiccation tolerant (Ellis *et al*., 1991). The germination in papaya takes place in 2-4 weeks after sowing. Seeds resulting from open pollinated flowers can produce plants with variation in sex types (a mix of male, female and hermaphroditic plants) which is very undesirable. Also, heterogeneity caused by cross-pollination is a drawback. Modern biotechnology offers various novel approaches for generating variability for crop improvement which can be applied to perennial fruit species where conventional breeding methods require a long time to attain the desired results. The techniques such as micropropagation, somatic embryogenesis, somaclonal variation etc. are beneficial tools for genetic manipulation of different crop species.

2.1 Chemical Constituents and Uses

Papaya is highly nutritive and rich source of vitamin A and C. Its latex has medicinal properties such as anti-bacterial and antifungal (Giordani and Siepai, 1991).It also contains four cysteine endopeptidases such as papain, chymopapain, glycyl endopeptidase and caricain. The constituents of *C. papaya* leaf extract showed twenty constituents, dominated by oleic acid (28.98%), with the least compound

Trans-Geranyl acetone (0.17%) (Oche *et al.*, 2017). The leaves are rich in elements like Ca2+, Mg2+, Na+ , K+ , Cl^- and Li+ (Vyas *et al*., 2014) which are essential elements and can be studied in detail for better medicinal uses with compared to the other parts of plant. The seed contain fatty acids, crude protein, crude fibre, papaya oil, carpaine, caricin, glucotropaeolin, benzyl glucosinolates, benzyl Isothiocyanate, benzyl thiourea, hentriacontane, ß-sitostrol and an enzyme myrosin (Kumar and Devi, 2017).The plant is also the source of two proteolytic enzymes, papain and chymopapain (Bhattacharya and Khuspe, 2001). These enzymes are commonly used for treating digestive or gastrointestinal tract disorders. Papain has diverse uses such as in food, beverage, and pharmaceutical industries which comprises production of chewing gums, drug preparations, chill-proofing beer, tenderising meat etc. A ready-to-serve papaya juice, candy or tutti-fruitti has been developed for bakery and confectionery industry at commercial scale in Coimbatore (TNAU, Coimbatore). Papain from immature fruits is used for degumming silk and for softening wool (Villegas, 1997).The fruit and seeds shows anthelminthic and anti-amoebic activities (Okeniyi *et al*., 2007).

Moreover, male and female plants have been found to be differing in the amount of the compounds produced. For instance, phenolic compounds tend to be higher in male plants than female plants. Also red-fleshed papaya fruit contain high levels of lycopene, whereas yellow-fleshed fruit do not (Chandrika *et al*., 2003). Kumar and Devi, (2017) reviewed about papaya that it promotes immune system, halt metastasis and can stop the growth of cancer cell. It also got anti-ulcer activity and is effective against hormone related to cancer. Review give details about aqueous seed extract of *C. papaya* at the dose of 50 mg/ kg and 100 mg/kg against gastric damage and blood oxidative stress in rats. The gastric acidity was considerably reduced in rats treated with 100 mg/kg of the extract. Moreover, aqueous extracts of leaves of papaya are given to dengue patients very frequently. Before giving the extract, the patient platelets count, white blood cells and neutrophils were decreased and the blood samples after giving leaves extract showed increased blood cells (Ahmad, 2011).

2.2 *In vitro* clonal propagation

Papaya cultivation is hindered by the inherent heterozygosity, dioecious nature (Bhattacharya and Khuspe, 2001) and susceptibility to viral diseases. As a result another efficient method of propagation of this plant is required for the production of specific sex types, disease free and true-to-type planting material. Plant tissue culture

is technique of growing and multiplication of plants on defined solid or liquid media under aseptic controlled environment. It can be achieved through axillary buds, cuttings, somatic embryos, cell clumps in suspension cultures (Gaikwad *et al*., 2017). It is a rapid proliferation method and one of the most efficient technique of applied biotechnology. As the world's population is increasing day by day and the facilities like space, agricultural lands are need to be increased simultaneously. So to cope with the ever increasing population, there is need of plant tissue culture which can ensure healthy and hunger free world for our next generation.

Micropropagation is an *in vitro* mass multiplication method of elite materials. Various attempts have been made to build up an alternate propagation method for production of quality planting material of papaya for better yield. Many factors control the *in vitro* multiplication of papaya plants like pH, genotype, epigenetic factors, explant, apical dominance, age of plant, nutrient medium and environmental conditions. The plan of this study was to achieve an improved multiplication method for production of *in vitro* raised papaya plants.

The pH of media shows significant effect on number of shoots produced on different treatments used in the experiment. Kanth, (2017) accounts for maximum number of shoots (10.83) at pH 5.7 while minimum number of shoots per explant (0.00) produced with pH 8.5 on MS medium. Also maximum shoot length (4.50 cm) was observed for pH 5.7 which was significantly at par with pH 6.0. Gelling agents are also known to control the growth response of cultures. Though, it is generally the cost and accessibility of the gelling agent that determines the type of material used. Gelling agents such as Gelrite® which is a water-soluble gellan gum, a polysaccharide derived from *Pseudomonas elodea.* This polysaccharide is comprised of glucuronate, rhamnose and cellobiose molecules and quantities of calcium, magnesium, potassium and sodium (George and Sherrington, 1984). However the cost of Gelrite® is higher than that of agar but effective at lower concentrations and thus proved cost effective. Agar is widely used water-soluble polysaccharide derived from the algae, *Gelidium* and *Gracelaria* species. The polysaccharide consists of repeating units of r3-D-galactose and a-L-anhydrogalactose, which are commonly substituted with methyl/sulphyl esters of pyruvate ketal groups (Wilson and Critchley, 1998). *In vitro* regeneration of papaya plantlets can be done using liquid and semi-solid/liquid double layer media with L-1 2 Chloro 4 Pyridy – N Phenyl-urea (CPPU) in double layer combination for shoot induction (Gatambia *et al*., 2016) and further

proliferation. McCubbin and Staden, (2003) reported that in papaya the rate of *in vitro* multiplication was affected by the type of gelling agent used. The multiplication rate in their study was reduced on gelrite with 3g /l activated charcoal whereas it gets higher on 1.5g/l activated charcoal and gelrite. Regeneration capacity of explants is also dependant on source of mother plants and there sterilisation. It is more aseptic if taken from greenhouse seedlings than from mature plants. Wu *et al.* (2012) treated seedlings with AgNO3 and had the 81.7% of contamination-free explants. Roy *et al.* (2012) used lateral buds and young leaves as explants with 84% plantlet survival in the green house. Further, axillary buds from hedged rooted cuttings were established on MS media 0.5mg/l 6-BAP, 0.1mg/l NAA (Reuveni *et al.*, 1990). A good proliferation using shoot tips on medium containing 5.0 µM NAA, 6BA, 10µM 2iP occurs for shoot development whereas 10 µM IBA showed 85% rooting with 80% survival rate.

Plant growth regulators (PGRs) in plant tissue culture have crucial for *in vitro* studies. PGRs mainly divided into five groups i.e. auxins, cytokinins, gibberellins, abscisic acid (ABA) and ethylene. Among PGRs, BAP and NAA combination at medium (0.5 to 2.5 mg/l) level concentrations for shoot initiation and multiplication shows encouraging results while IBA at lower and medium level concentrations for rooting were the best basal medium for *in vitro* propagation of papaya using shoot buds (Setargie *et al.*, 2015*).* Silva, (2016) reported that the auxins such as 2,4-D, dicamba and picloram generated callus and shoots were multiplied on BA and TDZ. Lai *et al*. (1998) formed plantlets of this plant on MS medium supplemented with 0.88 µM BA and 0.1 µM NAA. Yang and Ye, (1996) induced shoots from petioles on MS medium supplemented with Gamborg's B5 vitamins, 0.8 µM BA. (Tetsushi *et al.*, 2008). Agnihotri *et al.* (2004) observed plants were multiplied on 0.1 mg/l BAP, 0.25mg/l IAA, 40 mg/l adenine sulphate (AdS) and 50 mg/l L-glutamine with 80% transplant success. Geneve *et al*. (2007) reported 5 to 8 shoots on MS medium with or without PGRs using single shoot-buds. Various other researchers showed different reports regarding number of shoots developed. For example Ambasta and Kumari, (2013) admitted thirteen shoots on MS medium fortified with BAP 2.0 µM & NAA 0.5µM. Further Winnar *et al.* (1988) found MS modified media with 0.5 mg/l 6BA + 0.2 mg/l NAA best for establishment of shoot apices of cultivar Sunrise solo. Samanmalie (2017) sterilized shoot tips with 20% NaOCl for 20 minutes and used MS medium fortified with 3mg/l BAP+0.3mg/l NAA which resulted in enlargement

of explants in 3 weeks. Further confirmed by one-way ANOVA test with significantly higher treatment effect in the media containing 3mg/l BAP + 0.3mg/l NAA for shoot formation ($p= 0.0001< \alpha =0.05$) at commercial scale. For initiation and proliferation of papaya variety CO-5 cultures, the best medium was 0.50 mg l-1 BA along with NAA 0.10 mgl-1. *In vitro* rooting was then achieved with MS medium supplemented with 3.00 mg l-1IBA and activated charcoal 0.05 per cent (Bindu B, 2015).

Drew, (1987) observed synergetic effect of riboflavin on *in vitro* rooting. Teo and Chan, (1994) described that *in vitro* rooting in papaya produced thick, stumpy roots with basal callusing on full or half strength medium. Yu *et al*. (2000) observed rooting in darkness on MS medium fortified with 2.5 µM IBA and additional two weeks on half strength MS medium in aerated flasks. Drew *et al*. (1993) also demonstrated that 1g/l activated charcoal added to the media resulted in finer roots production and smaller shoots compared to those produced on a riboflavin treatment. After rooting, acclimatization of regenerated plants is necessary before being planted in the field. Pérez *et al*. (2015) explained that *in vitro* culture of papaya could be improved by increasing the ventilation in the vessel.

In papaya tissue culture endogenous bacteria is a major problem for *in vitro* multiplication of explants. Before detection for long-term production, a bacterial indexing procedure may be used to eliminate endogenous bacteria (Caple and Cheah, 2016). Papaya can be propagated under photoautotrophic conditions and LEDs which led to enhancement of leaf production than control plantlets (Silva, 2014). The number of shoots per culture can be increased further with the supplementation of additives along with plant growth regulators. According to Roy *et al*. (2012) additives like such as activated charcoal resulted in effective shoot growth with healthy leaf while urea in the medium leads to proper shoot elongation. Premature senescence of leaves during the *in vitro* culture of papaya is a major problem preventing the protocol to be used more often commercially. The closed vessel used in tissue culture leads to accumulation of gases in the vessel. Gases such as ethylene have been recognized by the researchers which cause yellowing and falling of leaves. For example, silver thiosulphate (STS) was used in nodal culture of papaya which increased leaf area and reduced leaf senescence (Magdalita *et al*., 1997). The amount of endogenous ethylene was lessened by adding inhibitors to the culture medium in the flasks. Exogenous ethylene in culture flasks was regulated using a gas diffusion equilibration procedure and flasks were sealed with 0.02 µm filters for diffusion and equilibration (Lai *et al*.,

2000). They observed an increase in shoot and leaf number with a regulated exogenous ethylene supply during the first week using a gas diffusion equilibrium procedure followed by an aeration of the flasks for the next two week which resulted in control of ethylene and increase both the quantity and quality of tissue available.

A papaya (*Carica papaya* L.) nodal culture grew poorly with leaves senesced because of ethylene accumulation in the headspace of the vessel. To estimate the ethylene accumulation, amino ethoxy vinylglycine (AVG), 1-aminocyclopropane-1-carboxylic acid (ACC) and silver thiosulphate (STS), were added to the nutrient medium. The ethylene-antagonist, STS significantly improved nodal culture growth and reduce the leaf senescence (Magdalita *et al*., 1997). Hence, micro-propagation could be better controlled by controlling ethylene accumulation and improved nodal cultures of papaya are obtained with shoot culture systems. The effect of aeration on development of multibuds to multishoots was also investigated by Lai *et al.* (1998).

2.3 Genetic fidelity

Variations occurring during *in vitro* propagation of plants are known as somaclonal variations (Larkin and Scowcroft, 1981). These are unpredictable and may be heritable or non-heritable (Saravanan *et al*., 2011). Somaclonal variations are associated with point mutations, DNA methyaltions, and transposable elements .These are reportedly influenced by the genotype, explant, culture conditions and age of plants (Jain, 1997). The amount of variation in the micropropagated plants might limit the commercial utilization of tissue culture system (Salvi *et al*., 2001). It will also effect the genetic fidelity of their clonal nature. Therefore, it is must to generate genetic uniformity among of *in vitro* raised shoots by using modern biotechnological methods.

Polymerase chain reaction based markers are random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR), are of immense importance in establishing genetic stability in *in vitro* propagated plants (Lakshmanan *et al*., 2007; Joshi and Dhawan, 2007). Homhuan *et al*. (2008) detected somaclonal variants in *Carica papaya* arising from indirect somatic embryogenesis using RAPD marker system. Kaity *et al.* (2008) reported somaclonal variation in cryopreserved shoot tips using PCR based randomly amplified DNA fingerprinting (RAF). Kaity *et al.* (2009) used both randomly amplified DNA fingerprinting and amplified DNA methylation polymorphism markers to assess genetic changes in developmental stages after cryopreservation.

Somatic embryogenesis is also an alternate method for the micropropagation which is lesser used until somaclonal variations are the need for the plant. But several results have been obtained. Somatic embryos may be induced from callus using petiole or internode segments. As per the various studies, auxins such as NAA, 2,4-D and IAA was important for the initiation and growth of callus. Chen *et al*. (1987) observe the regeneration of somatic embryos from seedling root in three months on ½MS containing 5.4 µM NAA+ 2.3 µM kinetin + 2.6 µM GA3,while Lin and Yang, (2001) done the same in four months adventitious roots. Callus was also induced from the midrib and lamina of cotyledons of *papaya* seedlings when cultured on MS basal medium (Litz *et al*., 1983). Bhattacharya and Khuspe, (2000) induced somatic embryos on MS medium conmprising 3 mg/L 2,4,5-T for 3-6 weeks. Davis and Ying, (2004) used Fitch's liquid medium for the induction of somatic embryos from immature seeds on while Magdalita *et al*. (2002) induced somatic embryos on de Fossard medium from zygotic embryos.

2.4 Genetic Diversity

2.4.1 Molecular Markers

Plant DNA isolation is a procedure to extract pure and intact DNA from the concerned plant genotype. The DNA extraction technique is employed in various research fields such as molecular biology, plant biotechnology, genomics, proteomics etc. The PCR based downstream applications also requires good quality and quantity of DNA (Devi *et al.,* 2013). In DNA extraction protocols the quality of DNA is restricted by biomolecules like RNA, protein, polysaccharides, polyphenols etc. and causes decreased yield of DNA. Therefore, separation of DNA from cellular components requires different enzymes and chemicals. The extraction buffer incubation with sample is the foremost step, followed by chloroform for phase separation and finally ethanol for precipitation for the DNA separation procedure. Xin and Chen, (2012), precipitate DNA by pellet re-suspension by bringing down the NaCl concentration in extraction buffer with CTAB dilution buffer. Ethylene diamine tetraacetic acid (EDTA) and cetyltrimethyl ammonium bromide (CTAB) inhibit DNase activity and 2M NaCl, was used to provide a high ionic strength reaction environment (Huang *et al*., 2013). Various secondary metabolites are present in plant species, they inhibit the reactions during isolation of DNA, which causes poor results with PCR-based genetic analysis. Sika *et al*. (2015) isolated DNA using SDS and high

salt concentration. It produced sufficient quality DNA for different downstream PCR-based analysis.

In Plant Biotechnology, molecular markers are used for various molecular studies, diagnostics, phylogenetic analysis, genetic studies etc. Some of the examples of such markers are RAPDs, ISSRs, SSRs, SNPs etc. which have been applied with various techniques. These are also used for high-throughput and ultra high-throughput level in DNA sequencing technique. (Grover and Sharma, 2014). The PCR-based markers are divided into two, the one without prior sequence information e.g., AP-PCR, DAF, RAPD, ISSR and other site-targeted with known DNA sequences e.g., EST, SSR, SCAR, STS) (Joshi *et al.,* 2000). SCARs (sequence characterized amplified region) are codominant markers which detect only a single locus (Paran and Michelmore, 1993). and used in the diagnostics. However, SNP has bi-allelic nature used for allele specific hybridization. Microsatellite markers are more suitable for diversity analysis and fingerprinting (Gurteg Singh, 2014). The markers for sex determination in plants include RAPD (Random Amplified Polymorphic DNA), SCAR (Sequence-characterized A-mplified Region), AFLP (Amplified Fragment Length Polymorphism), RFLP (Restriction Fragment Length Polymorphism) and microsatellites (Milewicz and Sawicki, 2013). Molecular markers enable estimation of the variations between individuals, regardless of their development stage (Sztuba- Solińska, 2005). The above applications have improved the fruit crop genetic resources and their utilization helps in crop improvement programmes. Therefore, molecular markers are valuable tools for assessing genetic diversity, species identification of several fruit crops such as papaya, apple, grapevine, peach, strawberry etc.

2.4.2 Genetic Polymorphism

Genetic variation is a key feature for survival, conservation and evolution of species. The germplasm of *Carica* show broad phenotypic difference in plant build, juvenile period, fruit size, shape and flavor (Drew *et al.,* 1998). The fingerprinting system created using RAPD and ISSR have been utilized for detecting genetic polymorphism in many horticultural crops for diversity analysis. The genetic diversity facilitates the identification of the various germplasms and cultivar improvement. Molecular markers are tools which benefit in identification of the individual polymorphism of cultivar of the plant. Huang *et al*. (2000) reported polymorphism in different papaya cultivar/varieties with RAPD markers. Kyndt *et al.* (2006) analysed Vasconcellea accessions with the help of 10 chloroplast and 9 nuclear SSR markers. In India the study on papaya diversity was done by Singh *et al*. (2006). They studied 21

accessions of papaya including Indian as well as exotic cultivars. The two varieties named 'Pusa Delicious' and 'CO 7′ was found to be good in growth attributes. The above study was surveyed with 20 SSR markers amoung 34 *Carica papaya* cultivars and seven species of Caricaceae genotypes. Using Jaccard's similarity analysis, Madarbokus *et al*. (2012) described the genotypic diversity existing in the four papaya varieties cultivated in Mauritius namely Farc, Long Orange, Ramnan and Uganda Female. The minimum and maximum similarity values were 0.28 and 0.806 using amplification with RAPDs markers. Rodriguez *et al*. (2010) reported genetic relationships between *C.cubensis* and *C.papaya* while Asudi *et al*. (2010) accessed 42 papaya germplasm available in kenya. They detected genetic similarity greater than 0.802. Oliveira *et al*. (2010a and 2010b) performed marker assisted selection of 83 papaya lines with 20 microsatellite primers, which produced an average of 3.18 alleles per primer. Pomper *et al.* (2003) reported high level of polymorphism in 19 papaya cultivars using 10 ISSR markers. Some researcher's (Ramos *et al.* 2012, Sudha *et al.* 2013) found that the qualitative and molecular data contributed to better understanding of the genetic relationships because of more accurate estimation of dissimilarity and genotype differentiation. Ramos *et al.* (2012) used Morpho-agronomic and molecular (RAPD and ISSR markers) data to evaluate genetic distances between thirty-two papaya backcross progenies for identification of agronomically superior genotypes. Sudha *et al.* (2013) characterized 73 andaman islands papaya genotypes using 24 ISSR primers and morphological diversity such as fruit weight, fruit length, fruit girth, flesh thickness etc. The primers produce 29.2% polymorphism with 37% similarity. Sengupta *et al*. (2013) studied genetic diversity using 20 SSR markers among 34 Indian *Carica papaya* cultivars and genetic similarity was in the range 7% to 67%. It showed separation of *Vasconcellea and Jacaratia spinosa* accessions from the *Carica papaya* accessions. Pinto *et al*. (2013) assessed 19 genotypes derived from JS12 and the Golden Sunrise'. This study used 20 polymorphic microsatellite markers for differentiation of parental genotypes. The major application of markers lies in the strategic research for rapid understanding of basic genetic mechanisms and genome organization at molecular level (Bhat *et al*., 2010). Warnakula *et al*. (2017) presented information on inter and intra specific relationships for C.papaya and mountain papaya using 11 SSR and 2 ISSR markers. They reported that papaya cultivars are not much divergent from each other because of the alleles found in the SSR and ISSR markers were same. Apart from above, chloroplast (cp) DNA markers were utilised to study 19

wild populations of papaya in Northern Mesoamerica (Pesqueira and Farfan, 2016). Clustering methods and the sum of rank index were used for analysis of genetic parameters related to the physiochemical and physiological characteristics of the seeds by Mengarda *et al*. (2015).

Papaya germplasm showed widespread morphological diversity in terms of fruit yield, weight, length, flesh thickness, flesh color etc. Ara *et al*. (2016) found that fruit length, yield and weight attributes of varieties were grouped in cluster III while cluster IV display plant height and pulp thickness of the varieties. These information would be helpful for the breeding program.

Jaccard's genetic similarity values helps in studies of diversity with RAPD and ISSR. Saran *et al*. (2015) found the similarity values in the range of 0.30–0.99 (average: 0.65) and 0.26–0.95 (average: 0.61) for RAPD, ISSR respectively. Dendrogram separated the selections into 4 main clusters and Lalima, Madhu, PS-3 were found better of other papaya germplasms. Sengupta *et al*. (2014) reported dendrogram with two major clusters, showed difference between Jacaratia spinosa and Vasconcellea accessions. The similarity between them ranged 1% to 53% only.

In other research, a total of twenty simple sequence repeat (SSR) primers were used to understand the genetic diversity between seven Indian papaya (*Carica papaya* L.) cultivars. Shivkumar *et al*. (2014) found polymorphic percentage of eighty with sixteen primers produced total of 42 alleles .The similarity coefficient ranged from 0.33 to 1.00 and was minimum (33 %) between Arka Prabhat and Co-5. Thus, dendrogram divided the genotypes into two broad clusters, Arka Prabhat, Honey Dew, Red Lady grouped into subcluster I and Solo and Arka Surya in subcluster II. Sudha *et al*. (2014) analysed genotypes using five ISSR markers and found similarity coefficient from 0.05 to 0.96.

2.5 Sex determination

Papaya is useful for genetic and evolutionary studies due to 372 megabases of genome. The female trees are stable in comparison hermaphrodite and male trees , which were reported to have sex reversals (Storey, 1976). Papaya trees display sexual polymorphism which is defined in 5-8 month old plants that either it is male, female or hermaphrodite. The farming of more female plants could be done, if female are identified at juvenile stage. Due to more female trees, space and water could be saved. Hence, the cultivation will be more economical or profitable.

Sex of plant is controlled by a single locus with three alleles — M1 (male), M2 (hermaphrodite) and m (female) (Somsri *et al.*,1998)). Male (M1m) and hermaphrodite plants (M2m) are heterozygous whereas female plants (mm) are homozygous recessive. Combinations of dominants which are, M1M1, M1M2, or M2M2 are lethal, M1m are viable males and viable hermaphrodites can only be M2m.The RAPD-SCAR marker system has been developed for the sex determination of papaya varieties. Diversity in papaya cultivars needs to be exploited for developing superior cultivars for characteristics like better fruit quality and disease resistance. The breeding in papaya could be made more beneficial using SCAR marker, for sex determination and which helps in reducing expenses of the breeder. It will be useful to the farmer and good for commercial value.

2.5.1 Molecular markers for identification of sex type

At present, molecular markers are valuable tools for determining the sex at seedling stage in papaya (Deputy *et al*., 2002). The proficient marker should guarantee reproducibility and also be easy to detect. The markers differentiate between genotypes based on their genetic variations (Sztuba- Solińska, 2005). Therefore various attempts have been made to determine the sex of papaya plants using molecular markers. In papaya, RAPD and microsatellite markers linkage to sex has been observed (Sondur *et al*., 1996). Somsri *et al*. (1998) identify male-specific bands using random amplified polymorphic DNA (RAPD) and DNA amplification fingerprinting (DAF). RAPDs markers have also been used by Lemos *et al*. (2002) and Urasaki *et al*. (2002a, 2002b) for sex type identification in papaya. Somsri and bakornkul, (2007) used 52 primers in bulk segregate analysis (BSA) and found male and hermaphrodite specific bands. Gangopadhyay *et al*. (2007) used RAPDs and ISSR marker for the determination of sex of the papaya plants. Parasnis *et al*. (2000) developed sex-linked markers, using sequence-characterized amplified region (SCAR) which were tightly liked to sex forms. Deputy *et al*. (2002) detect bands specific to hermaphrodite and male plants in papaya varieties Sunrise and Kapoho using SCAR markers W11 and T12. Similarly Giovanni and Victor, (2007) and Niroshini *et al*. (2008) reported sex detection at seedling stage of papaya. The sex determination in papaya, identified fruit bearing plants and it can be done using molecular markers, which provide identification of sex at initial stages only. Chaturvedi *et al*. (2014) validated W11 (SCAR) marker for male and female plants using PCR. However, Soni *et al*. (2017) recognized male and female palnts by

observing three-lobed /five-lobed leaves of the papya. The overall papaya farming has a major risk from several fungal and viral diseases. The female and hermaphrodite plants bear fruits and which plant will produce fruit is not sure until flowering. The multiplex PCR based detection method is consistent and cost-effective. Many researchers such as Yoke-Kqueen *et al.* (2011), Nageswara-Rao *et al.* (2013) reported use of multiplex to identify GMOs (Genetically Modified Organisms).

Alahakoon *et al*. (2013) found the approximate cost to predict the sex of seedlings is Rs.500/- per seedling. They receive leaf samples from growers and predicted within six hours to know the sex of their future plants and adjust the female to male plant ratio without unnecessarily wasting resources on unproductive plants. Reddy *et al.(2012)*revealed distinct polymorphism between the plants of three lobed and five lobed leaf types using 20 RAPD primers.

For increasing the papaya yield, the ratio of female to hermaphrodite plants could be 1:2. Identification of sex of seedlings during preliminery stage is of prime significance. Sobir *et al.* (2008) used five SCAR markers to examine 24 genotypes of papaya from 12 populations and differentiated male and hermaphrodite plants using primer PKBT-5.