SUMMARY AND CONCLUSIONS

Fruits contain many components of food such as proteins, vitamins, minerals, carbohydrates etc. with additional enzymes and secondary metabolites. Papaya (*Carica papaya* L.) belongs to family Caricaceae and it is native to Central and South America. It is propagated in tropical and subtropical regions of the world with climatic conditions like 22-26°C temperature, 100-150 cm rainfall. The plants are soft-wooded and fast-growing with a height upto 3-6 meters.

Conventional propagation of papaya is hindered by its heterozygosity, dioecious habit and susceptibility to viruses. Thus, to fulfil the demands of elite planting material, alternative methods of propagation are required. Therefore the present investigation was conducted on "Micropropagation, sex determination and assessment of genetic diversity in *Carica papaya*". The differentiation of sex type of a particular papaya seedling would be advantageous, since the desired sex type can be selected prior to micropropagation. Thus the plants obtained are either females or hermaphrodites depending upon the mother plant utilized. The regenerated plants were analysed using RAPD and ISSR markers for checking the somaclonal variations. The present investigation of seedling has been identified using SCAR and ISSR markers and was validated in five dioecious papaya varieties. Lastly the genetic diversity has been assessed among 18 different genotypes of papaya using RAPD and ISSR markers.

- For micropropagation, plants were surface sterilized with 0.1% $HgCl_2$ for different durations. The maximum (44.4%) survival of nodal explants was recorded on treatment ST4 when nodal explants were treated with 0.1% $HgCl_2$ alone for 4 min.
- The percentage survival was reached maximum (100%) when treated with antibacterial and antifungal agents. The response of nodal segment was found best on eight media combinations PE_2 (MS basal + 0.5 mg/l BAP), PE_8 (MS + 1.0 mg/l kinetin), PE_{15}(MS + 4.0 mg/l GA_3), PE_{17}(MS + 0.5 mg/l BAP+0.1 mg/l NAA), PE_{18}, PE_{19}, PE_{24} and PE_{33}.

- The maximum (88.8%) survival percentage was reported when nodal explants were treated with bavistin (0.4%) along with streptocyclin (0.4%) for 120 min followed by $HgCl_2$ (0.1%) for 4min.
- The minimum survival percentage (22.2%) was reported on treatment ST5 when nodal explants were treated with 0.2% bavistin and 02.% streptocyclin for 60 min. prior to the treatment of $HgCl_2$ (0.1%) for 4min.
- Thus, nodal segments showed better survival and lesser contamination at higher concentration (0.4%) and durations (120,150 min.) of streptocyclin and bavistin for survival percentage. The above treatments also reduced *in vitro* culture contamination due to endophytic bacterial problem in papaya.
- The surface sterilized nodal segment were cultured on Murashige and skoog basal medium with growth regulators viz. BAP, KIN, TDZ, NAA, IBA, Zeatin and GA_3. The maximum (100%) response of nodal was observed on eight media combinations PE_2 (MS basal + 0.5 mg/l BAP), PE_8 (MS + 1.0 mg/l kinetin), PE_{15} (MS + 4.0 mg/l GA_3), PE_{17} (MS + 0.5 mg/l BAP+0.1 mg/l NAA), PE_{18}, PE_{19}, PE_{24} and PE_{33}.
- Out of these eight media combinations PE_{18} (MS + 0.5 mg/l BAP+0.1 mg/l NAA) medium was most effective with 100% response and 6.4 buds/explant in 9.0 days followed by (88.8 %) response observed on media PE_{16}, PE_{32} and PE_{44} with 2.5, 2.0, 3.3 buds/explants in 11.3, 12.3, 13.3 days respectively.
- The lowest percentage response (22.2%) was observed on PE_{45} and PE_{50} media in fifteen days.
- The best effective medium out of alone BAP, KIN and GA_3 was in BAP (0.5 mg/l) with 5.1 buds/ in 9.6 days, followed by GA_3 (4.0 buds/explants in 11 days) and KIN (3.7 buds/explants in 12 days).
- The well-established plants were shifted to MS media for shoot multiplication of papaya supplemented with 50 combinations of plant growth regulators (PGR) such as BAP, GA3, NAA, IBA, IAA, KIN, TDZ and Zeatin.
- According to one-way ANOVA results, a significantly higher treatment effect was recorded for M8 group, which produced maximum (15.7) no of shoots on PM_{38} (1.0 mg/l Kinetin and 0.3 mg/l NAA) medium.
- The mean number of shoot obtained for all other groups in decreasing order are as M6, M1, M7, M5, M2, M4, M9, M3 and M10 with 15.0, 14.6, 13.4, 12.7, 11.2, 6.7, 6.7, 5.8 and 5.6 shoots respectively.

- The media combinations which produce more than thirteen shoots during multiplication were PM_{27}, PM_4**,** PM_{28} and PM_{34}, whereas the lowest mean no of shoots were as low as 2.3 on PM_{46} and $PM_{50.}$medium on 28th day of culturing. The addition of GA_3 with auxin and cytokinin in group M6 produced best results for all concentrations and all combinations produced maximum (11.3-15.0) no. of shoots per culture.
- The varying concentrations of additives viz. adenine sulphate, polyethylene glycol, putrescine, silver nitrate and spermidine were supplemented with BAP (0.5mg/l) and GA_3 (4.0 mg/l) with MS basal (PMA_1-PMA_{25}) medium.
- The mean comparison test for number of shoots and percentage leaf fall showed a significant effect on leaf senescence for the PMA_1, PMA_2 and PMA_3 medium.
- The PMA_2 media showed the highest mean no of shoots i.e. 18.9, followed by 17.1(no of shoots) on PMA_3 media, at the same time percentage leaf fall were13% and 19% respectively.
- The lowest number of shoots was recorded 3.2(PMA_{25}) on the additive spermidine, followed by putrescine, PEG and adenine sulphate in increasing order of number of shoots.
- Rooting and hardening results shown that the R3 (2.5 mg l-1 IBA) media develop 100% root induction while R4 showed 88% response after 21 days of culture.
- The highest survival percentage for hardening was 80.0 % followed by 70% in sand, soil and FYM. The least survival of plantlets was observed in only sand containing potting mixture.

The ISSR and RADP primers confirmed that the plants cultured in *in vitro* conditions were true-to-type.

- Fifteen ISSR primers produced clear and scorable bands. A total of 67 bands with an average number of 4.6 were imaged ranging between 250 to 1500 bp length.
- Similarly, Fifteen RAPD primers amplified and imaged to show a total of 81 bands ranging between 200 to 3500 bp length. The average numbers of bands were 5.4 per primer.
- Maximum (10) number of bands in ISSR was obtained with primer AY21 and RAPD5 produced maximum (11) number of bands amoung RAPDs.

For sex determination the known mature male and female DNA samples and the unknown seedling DNA of papaya was purified for validation.

- The SCAR1 primer produced male specific bands in Pant2 and Pusa dwarf varieties, but it failed to amplify DNA from female plant.
- Similarly, primers SCAR 2 and SCAR3 also produces male specific band in three varieties each. SCAR2 was validated for Pusa nanha, Pusa dwarf and Pant papaya while pusa nanha, pant papaya and pant2 for SCAR3.
- The primer $(GACA)_4$ amplified both male and female DNA samples of papaya.
- The screening was performed with known male and female dioecious varieties of *Carica papaya*. These known samples were then used for validation experiment.

The PCR amplification was carried out for 18 papaya varieties with RAPD and ISSR markers to generate DNA profile for assessment of genetic diversity.

- Twenty primers produced 103 bands, 45 were monomorphic and 58 were polymorphic. The number of scorable bands for each RAPD primer ranged from 2 to 10 with an average of 5.1 bands per primer.
- The maximum ten bands were produced by primer OPB-07 and OPA-13 whereas minimum two bands were produced by OPB-15.
- The percentage polymorphism varies within 20-100% with an average value of 54.5 % amongst cultivar.
- Primer OPA-1, OPA-16 and OPB-15 showed 100% polymorphism while OPA-2, OPA-6 showed 25% and 20% polymorphism respectively.
- The PIC values of primers ranged between 0.02-0.52 and an average was found to be 0.22 per primer.
- The primer OPH-03 was best of all other primers because of highest (0.52) PIC value followed by RAPD 5, OPG-03, OPG-02 etc. Overall size of amplified products ranged between 200 bp and 2500 bp.
- The 20 ISSR produced 100 distinctive bands among 18 papaya varieties. Out of 100 bands, 48 were monomorphic and 52 were polymorphic.
- The ISSR primer bands ranges from 1 to 11 having average of 5.0 bands per primer. The maximum number of bands was produced by primer AY-21 followed by AY-27 whereas minimum one band was produced by AY-31, AY-32.

- The percentage polymorphism varies within 25-100% with an average value of 47.2 % amongst cultivar. Primer AY-45 showed 100% polymorphism while AY-13, AY-26 showed 25% and 37.5 % polymorphism repectively.
- The PIC values of primers ranged between 0.02-0.52 and an average was found to be 0.20 per primer.
- The primer AY-6 was best of all other primers because of highest (0.52) PIC value followed by AY-37, AY-29,AY-45 etc.

The NTSYS-pc UPGMA cluster analysis was done using genetic similarity and led to the dispersal of 18 papaya varieties, into three major groups.

- The major group one is further divided into two subgroups with subgroup I having one papaya genotype P1. The subgroup II has P16, P17 and P18 papaya genotypes.
- The major group II was also divided into two subgroups with subgroups 1 having P2, P3, P4, P5, P7 and P6 genotypes and other subgroup having P14 and P15 genotypes.
- The major group III was divided into two subgroups with subgroup 1 having one genotype P8 and other subgroup having five genotypes such as P9, P10, P11, P12, P13.
- The range of similarity coefficient was found within 0.67 to 0.95. The maximum value in similarity index was noted for four varieties in the II major group which are Pusa majesty and Pune selection; PS1 and Pusa dwarf.

Dendrogram created from the combined data from RAPD and ISSR showed similarity range from 0.67-0.95.

- Group I includes four varieties of papaya i.e. Pant 2 in one subgroup and meerut, CO2, Pant in the other. Among these four, CO2 found most similar with pant papaya with 94 % similarity and both CO2 ,Pant papaya were least similar with Pusa Giant with 67 %, 69 % similarity respectively.
- The Pant 2 was similar to other varieties in the group with an average of 80% but maximum similarity (88 %) in the index was shown with Pusa majesty.
- Group II includes eight varieties of papaya with Pusa nanha and sonepat in one subgroup with 91% similarity while other subgroup includes Pusa majesty, Pune selection, PS1, Pusa dwarf, Pusa delicious and Pusa giant.

- The maximum similarity of 95% was found between Pusa majesty, Pune selection and PS1, Pusa dwarf. The above four varieties were least similar (71%) with Sirsa.
- Group III showed six papaya varieties with two subgroups. The unknown variety from sirsa region was most similar with Washington with 83% similarity and least similar with PS1 with 71% similarity.
- The unknown variety from hisar was most similar with 92% similarity with Tripura selection. Also unknown sample from Delhi was found most similar with CO7 having 93% similarity.

Thus the protocol obtained in present study, could be used for commercial propagation of papaya. Before propagation of plants, the seedlings test for sex type could be done using SCAR markers, identified in the present study. The diversity results show lesser polymorphism among varieties. The similarity values obtained was higher, depicting less divergence among varities.