Brinjal (*Solanum melongena* L.) belong to the *Solanaceae* family with the diploid chromosome number 2n = 24. The experiment entitled on “Morphological, Biochemical and Molecular Characterization of Brinjal (*Solanum melongena* L.) Genotypes Through PCR Based Molecular Markers” was conducted at Biotechnology Laboratory, Department of Genetics and Plant Breeding, Junagadh Agricultural University, Junagadh during the year 2015-16, to find out genetic diversity at morphological, biochemical and molecular marker levels. The experimental material comprised total of 12 brinjal genotypes.

At present, morphological features are commonly used to differentiate brinjal cultivars. However, characterization based on phenotypic trait is unreliable since they can be affected by environmental conditions. Isozymes have proven to provide simple and reliable markers as electrophoresis of plant enzymes is more rapid than field-testing and can be detected with a small amount of plant tissue extracts. It provides a valid biochemical basis of varietal identification and may be used as legitimate evidence of novelty. Compared to morphological and biochemical markers, molecular markers are noteworthy because they are unaffected by environmental changes or growing stages, detects more variation, do not change the morphology and simply inherited. Among DNA based markers, RAPD, ISSR and SSR are useful for molecular characterization and have large number of applications like characterization of gene pool, DNA fingerprinting, phylogenetic analysis, molecular dissection of complex traits, and characterization of genome organization. Keeping this in view and to check whether this technique could reflect diversity among the genotypes, the experiment based on morphological, biochemical and molecular and biochemical markers was conducted with 12 brinjal genotypes.

Twelve qualitative characters were studied in 12 brinjal genotypes. Genotypes JBL-08-08, LB-12-06, Swarna Mani Black and GJB-2 showed prostrate growth habit, while Swarna Mani Black showed violet stem colour, JBL-08-08, NSR-1, KS-224, Pant Rituraj, Swarna Mani Black, GJB-2 and GJB-3 showed strong leaf blade lobing. In case of leaf blade length JBL-10-208 and JB-12-06 showed long leaf blade as...
compared to others, while three genotypes named AB-09-01, JBL-10-11 and JBL-10-208 had wide leaf blade width. In case of leaf blade tip angle JBL-08-08 and KS-224 had acute leaf blade tip, while AB-09-01 display light violet corolla colour. Genotype JBL-08-08 has slight curved and long fruit shape, while JBL-10-11 showed striped and oblong fruit shape. Fruit flesh density was highest in Swarna Mani Black and JBL-08-08 displayed grayish yellow seed colour which was quite different as compared to other genotypes.

Among twelve genotypes of brinjal, seven quantitative characters were determined at morphological level. The highest plant height was observed in PLR-1 and lowest in GJB-3. The highest plant spread, 10-fruit weight, fruit length, fruit girth, fruit length/girth ratio and 100-seed weight were recorded in PLR-1, GJB-3, JBL-08-08, JBL-08-08, JBL-08-08, and JB-12-06, respectively, while lowest plant spread, 10-fruit weight, fruit length, fruit girth, fruit length/girth ratio and 100-seed weight plant h plant spread, 10-fruit weight, fruit length, fruit girth, fruit length/girth ratio and 100-seed weight were observed in JB-12-06, JB-12-06, JB-12-06, JBL-10-208, JBL-10-208 and KS-224.

The clustering pattern of 12 genotypes for quantitative traits showed that the genotypes of different origins were clubbed in one cluster, whereas the genotypes belonging to same origin were grouped into different clusters indicating that the geographic distribution did not consider as sole criterion of genetic diversity. The maximum inter cluster distance (D=28.13) was observed between cluster I and cluster IV followed by that between cluster II and cluster III (D=22.90) and between cluster III and cluster IV (D=22.20). The minimum inter cluster distance was observed between cluster I and cluster II (D=19.29). The genotypes belonging to the clusters separated by high statistical distance could be used in hybridization programme for obtaining a wide spectrum of variation among the segregates or to exploit maximum level of hybrid vigour in brinjal. In this context, genotypes from cluster I (JBL-08-08, AB-09-01, JBL-10-011, JBG-10-208, NSR-1, KS-224, Pant Rituraj, Swarna Mani Black and GJB-2), cluster II (PLR-1) cluster III (GJB-3) and cluster II (JB-12-06) should be selected as parents in hybridization programme.
Isoenzymes and biochemical activities were used for the characterization of 12 brinjal genotypes. The maximum Peroxidase activity recorded by KS-224 and minimum activity showed by JBL-10-011. GJB-2 had minimum total sugar content, reducing sugar content and antioxidant activity. In case of Esterase activity JBG-10-208 was on top among all the genotypes, while genotype AB-09-01 showed highest and Pant Rituraj showed lowest Polyphenol oxidase activity. Genotype AB-09-01 had lowest chlorophyll, carotenoids and protein content. In case of Phenol content, genotype PLR-1 showed maximum and GJB-2 showed minimum values, while Superoxide dismutase activity rate is low in genotype Pant-Rituraj and high in NSR-1. Glycoalkoloid content was very high in three genotypes namely JBL-10-208 and low in genotypes JBL-08-08, AB-09-01 and JB-12-06.

In case of isoenzymes, Peroxidase generated four bands at 10 DAG. Out of four bands, two was polymorphic having 0.26 and 0.58 Rm values respectively. Esterase generated three bands, out of three one band (0.64 Rm value) was polymorphic. Polyphenol oxidase generated five bands out of which three bands were polymorphic having 0.3, 0.58 and 0.73 Rm values. Superoxide dismutase generated six bands out of which two bands were polymorphic having 0.48 and 0.65 Rm values.

The genetic diversity among 12 brinjal genotypes were studied using RAPD, ISSR and SSR markers. Total genomic DNA was extracted from leaf tissue of twelve genotypes using Doyle and Doyle (1987) with minor modifications as per Mohammed et al. (2012). All the DNA samples were of good quality and quantity. The yield of DNA isolated ranged from 1314.75 ng/µl in JBL-08-08 to 4838.66 ng/µl in KS-224 with optical density near about 1.7 to 2.3 indicated that DNA extracted was pure in all the genotypes.

Seventeen RAPD primers generated total of 232 bands/alleles in which 224 bands were polymorphic showing 96.55% polymorphism. The average bands per primer were 13.17. The polymorphic information content (PIC) was recorded from 0.7947 to 0.9405. The highest PIC value of 0.9405 was recorded by OPC-17, while the lowest PIC value of 0.7947 was recorded by OPB-20. Similarly RAPD primer index (RPI) ranged from 3.9739 to 18.819 with an average of 12.4250 bands per primer. The highest RPI value was obtained by OPC-17 and the lowest was obtained by OPB-20. Jaccard’s coefficient of similarity of 12 brinjal genotypes ranged from 27.9% (JBL-...
SUMMARY AND CONCLUSIONS

08-08) to 59.3% (between JBL-10-011 and JBG-10-208). The phylogenetic tree constructed by UPGMA method generated two main clusters which again sub-grouped in their respective sub-clusters.

Seventeen ISSR primers produced 161 bands/alleles in which 148 bands were polymorphic and 91.92% polymorphism with an average of 8.71 bands per primer. The polymorphic information content (PIC) ranged between 0.7409 and 0.9008. The highest PIC value of 0.9008 was noticed in ISSR-59, while the lowest PIC value of 0.7409 was noticed in UBC-842 with an average of 0.8557 per primer. Likewise, ISSR primer index (RPI) ranged from 3.7045 to 13.512 with an average of 8.193 per primer. The maximum RPI value was obtained by ISSR-59 and the minimum was obtained by UBC-842. Jaccard’s coefficient of similarity between 12 brinjal genotypes ranged from 37% (between Pant-Rituraj and JBL-10-01) to 63% (between JBG-10-208 and NSR-1). The cluster analysis of ISSR revealed the two main clusters, which further divided into various sub-clusters.

Fourteen SSR primers produced 33 bands/alleles in which 30 bands were polymorphic and 90.91% polymorphism with an average of 2.14 bands per primer. The polymorphic information content (PIC) ranged between zero and 0.7372. The highest PIC value of 0.7372 was noticed in Sgn/E514602, while the lowest PIC value of zero was noticed in SmSSR-11, SmSSR-12, SmSSR-15, EEMS-15 and Sgn/E514601 with an average of 0.3756 per primer. Likewise, SSR primer index (RPI) ranged from 0.0 to 3.657 with an average of 1.2188 per primer. The maximum RPI value was obtained by EM-140 and the minimum was obtained by SmSSR-11, SmSSR-12, SmSSR-15, EEMS-15 and Sgn/E514601. Jaccard’s coefficient of similarity between 12 brinjal genotypes ranged from 20% (between Pant-Rituraj and PLR-1) to 87.5% (between Swarna-Mani-Black and GJB-2). The cluster analysis of SSR revealed the two main clusters, which further divided into various sub-clusters.

The pooled study of molecular marker through RAPD, ISSR and SSR was done to confirm the differences and similarity between 12 brinjal genotypes. Dendrogram developed by Jaccard’s similarity coefficient and UPGMA method showed the highest (60.8%) similarity between JB-12-06 and JBG-10-208 and the lowest (32.0%) similarity between JBL-08-08 and Swarna Mani Black. The dendrogram consisted of two main clusters I and II with an average similarity of 38%.
Among the studied techniques, RAPD primers gave slightly higher polymorphism among the genotypes (96.55%) as compared to ISSR markers (91.92%) and SSR markers (90.91%). More number of polymorphic bands, more PIC and higher percentage polymorphism per primer was amplified by RAPD as compared to SSR and ISSR markers. RAPD, ISSR and SSR markers gave distinct clustering patterns.

Based on molecular data in the present study it can be concluded that the molecular markers could be a better tool for studying the genetic diversity. The genetic diversity analysis through biochemical and molecular markers resulted in developing moderate diversified map of 12 brinjal genotypes. However, the biochemical techniques were poorly distinguished genotypes as compared to techniques of DNA fingerprinting. Therefore, the molecular techniques are more accurate and more reliable than biochemical markers.

1. Twelve qualitative traits studied revealed moderate genetic diversity in 12 brinjal genotypes distributed nine genotypes in cluster I and solitary genotype in cluster II, cluster III and cluster IV each. Likewise seven quantitative traits revealed similar clustering pattern to that of qualitative traits.

2. Among biochemical parameters, genotypes KS-224, JBG-10-208, AB-0901 and NSR-1 showed maximum peroxidase, esterase, PPO and SOD activity, respectively. Very high glycoalkaloid was observed in JBL-10-208 followed by high glycoalkaloid in JBL-10-11 and GJB-2. Likewise, the genotype JBL-08-08 showed maximum chlorophyll-A and total chlorophyll; NSR-1 gave maximum total sugar; Pant Rituraj gave maximum carotenoids; PLR-1 showed maximum protein and finally GJB-2 gave maximum reducing sugar, antioxidant activity and phenol content.

3. Seventeen RAPD primers selected in the present study gave 96.55% polymorphism. Genetic diversity analysis through RAPD marker gave highest (100%) polymorphism percentage with primers viz., OPA-05, OPA-14, OPA-15, OPA-16, OPA-18, OPB-18, OPB-20, OPC-17, OPD-12, AC-14, AY-12 and OPN-05. Therefore, these primers were most useful for genetic diversity analysis to generate DNA fingerprinting in brinjal genotypes.
4. Seventeen ISSR primers selected in the present study gave 91.92% polymorphism. The highest polymorphism percentage (100%) was observed with primer UBC-807, UBC-810, UBC-813, UBC-812, UBC-820, UBC-823, UBC-900, UBC-09, ISSR-40 and ISSR-59. Therefore, these ISSR primers can be used further for diversity study in different brinjal genotypes.

5. SSR primers selected in the present study gave 90.91% polymorphism. The highest polymorphism percentage (100%) was observed with primer smSSR12, smSSR15, smSSR16, EEMS15, sgn|E514601, sgn|E514602, sgn|E514647, sgn|E513947, sgn|E515884, EM140 and EM145. Therefore, these SSR primers can be used further for diversity study in different brinjal genotypes.

6. In pooled analysis of molecular marker revealed the formation of several subclusters within cluster II suggested the presence of moderate genetic diversity among the 12 brinjal genotypes studied. The geographical diversity was not associated with genetic diversity. The highest 60.8% Jaccard’s similarity was observed between JBL-12-06 and JGB-10-208 and the lowest 32.0% similarity between JBL-08008 and Swarn Mani Black.