CHAPTER - V
SUMMARY AND CONCLUSION

The experiment entitled “MOLECULAR CHARACTERIZATION OF BLACKGRAM [Vigna mungo (L.) Hepper] GENOTYPES FOR SALINE WATER TOLERANCE” was conducted at Department of Biotechnology, Junagadh Agricultural University, Junagadh during 2017-2018, the results are summarized and concluded in this chapter. The experimental material was comprised of 22 blackgram genotypes and they were screened against different salinity levels for saline water tolerance and susceptibility based on physiological parameters. After screening, twenty genotypes were studied for polymorphism and the genetic diversity among saline water tolerant and susceptible blackgram genotypes using DNA based molecular markers (RAPD, ISSR and SSR). The physiological changes were observed under saline water stress conditions using various physiological parameters. The results are summarized and concluded in this chapter.

5.1 PHYSIOLOGICAL OBSERVATIONS

Physiological observations like germination percentage, total root length, total shoot length, seedling length, seedling dry weight and vigour indexes were observed in the present experiment. The salt treatments, $T_1$ (control) $T_2$ (ECe 4 dSm$^{-1}$), $T_3$ (ECe 6 dSm$^{-1}$), and $T_4$ (ECe 8 dSm$^{-1}$) were given to the seeds in Petri dishes in the laboratory condition. All the physiological observations were taken at the 10 days after sowing. Vigor Index-I and vigor index-II were calculated based on length and dry weight of seedlings. The blackgram genotypes were categorized as salinity tolerant, moderately tolerant and susceptible according to their performance under salinity treatment. Looking to the data on various physiological parameters, genotypes SKNU-03-03, SKNU-07-06, SKNU-06-03, SKNU-07-01 were found as salinity tolerance and GJU-1506, JAWAHAR URD-3, JAWAHAR URD-2 and GJU-1509 were found to be salinity sensitive genotypes. All other remaining genotype are categorized as moderately tolerant genotypes against salinity.
Summary and Conclusion

In all the genotypes, germination percentage, total root length, total shoot length, seedling length, seedling dry weight, vigour index-I and vigour index-II were found to be decreased as salt concentration increased and all the parameters showed minimum values in the T4 (ECe 8 dSm⁻¹) treatment as compared to all other treatments.

At high saline water concentration (ECe 8 dSm⁻¹), mean germination percentage (67.77 %) found maximum in IC-14520 (90 %), followed by genotypes IC-204869 (89 %) while, the highest vigor index-I (1833.69 mg) in genotypes JAWAHAR URD-3 these genotypes showed their salt tolerance capacity according to their performance under salinity stress. The minimum value for germination percentage (38 %) was observed in SKNU-03-03 genotypes, while, seedling vigor index-I was minimum (320.54 mg) in SKNU-03-03 genotype under maximum salinity level (ECe 8 dSm⁻¹) which showed its salt susceptibility. Vigour index-II found maximum (1081.75) in genotype SKNU-9915 while, it is minimum (670.30) in genotype TPU-94-2.

Under maximum salt concentration (ECe 8 dSm⁻¹) the root length was found highest (8.89 cm) in genotype JAWAHAR URD-3 followed by GJU-1506 (8.39 cm) which showed their salinity tolerance, while, lowest (2.32 cm) recorded in salinity susceptible genotype SKNU-03-03 at high salinity level (ECe 8 dSm⁻¹) might be due to its salt sensitivity.

At high salinity level (ECe 8 dSm⁻¹) the shoot length was found highest (13.39 cm) in genotype JAWAHAR URD-3 while, lowest (4.40 cm) value was recorded in genotype SKNU-06-03.

The seedling length was found highest (23.3 cm) in genotype JAWAHAR URD-3 followed by genotypes GJU-1506 (20.5 cm) which were categorized as salinity tolerant genotypes, while, lowest value was observed in the genotype SKNU-03-03 (6.9 cm) at high salinity level (ECe 8 dSm⁻¹).

At high salinity level (ECe 8 dSm⁻¹), the seedling dry weight was found highest as 14.34 mg in genotype SKNU-03-03 and lowest seedling dry weight 6.04 mg in genotype TPU-94-2.
5.2 MOLECULAR CHARACTERIZATION

RAPD Analysis

Total 11 RAPD primers were amplified which generated total 81 fragments out of which 69 bands were polymorphic with an average of 7.27 bands per primer and 1 bands were monomorphic. The PIC (Polymorphism Information Content) values were varied from 0.50 (OPA-20) to 0.91 (OPA-02) with an average of 0.78 per primer while the RPI (RAPD Primer Index) differed from 0.56 (OPY-15) to 13.65 (OPA-02) with an average of 5.67. Out of 11 RAPD primers, five primers were able to produce genotype specific unique bands viz., OPA-02 (band size 572.8, 417.7 and 628.6 bp in IC-14520, IC-204869 and SKNU-06-03 ), OPA-05 (316.3 bp size band in IC-61100), OPA-07 (band size 2341 and 2654 bp in COBG-593 and TPU-4), OPA-13 (band size 536 and 618 bp in SKNU-07-03 and SKNU-06-03), OPA-18 (band size 1597 bp in SKNU-06-03), OPA-20 (band size 3058 bp in SKNU-07-06) and OPE-19 (316 bp size band in IC-242677). The phylogenetic tree constructed by UPGMA method generated two main clusters and similarity coefficient was ranged from 10 to 68 %. SKNU-07-03 and SKNU-03-4 with similarity 68% (Most similar), and most diversified genotype was SKNU-07-01.

ISSR Analysis

Total 10 ISSR primers produced total 47 fragments in which 44 bands were polymorphic with an average of 4.40 bands per primer and 3 bands were monomorphic. The PIC (Polymorphism Information Content) values were varied from 0 (UBC-821) to 0.99 (UBC-811) with an average of 0.65 per primer while, the IPI (ISSR Primer Index) ranged from 0.00 (UBC-821) to 6.93 (UBC-811) with an average of 3.46. Out of 10 ISSR primers, five primers produced genotype specific unique bands viz., UBC-808 (1296 bp size band in SKNU-03-04), UBC-817 (331 bp size band in SKNU-07-01), UBC-819 (789 bp size band in GJU-1506) and UBC-830 (222 bp and 630 bp size band in SKNU-07-01COBG-593) were able to produce genotype specific unique bands to identified blackgram genotype. The phylogenetic tree constructed by UPGMA method generated two main clusters and similarity coefficient was ranged from 35 to 90 %. IC-14520 and IC-214845 with 90 % similarity (Most similar), and most diversified genotype was JAWAHAR URD-3.
SSR Analysis

Total 10 SSR primers generated total 14 fragments out of which all 14 bands were polymorphic while no, monomorphic band were produced. The Polymorphism Information Content (PIC) values for SSR markers were ranged from 0.00 (CEDG10, CEDG86, CEDG92, CEDG258, CEDG275, CEDG284 and CEDG291) to 0.48 (CEDG43) with an average value of 0.12 per primer and SSR primer index (SPI) differed from 0.00 (CEDG10, CEDG86, CEDG92, CEDG258, CEDG275, CEDG284 and CEDG291) to 1.44 (CEDG43) with an average value of 0.28. Out of 10 SSR primers any did not show the amplification of unique band and blackgram genotype specific bands in different genotypes. The phylogenetic tree constructed by UPGMA method generated two main clusters and similarity coefficient was ranged from 40 to 100%.

The collective data of RAPD, ISSR and SSR markers used to generate mixed clustering pattern to confirm the difference and similarity between genotypes as the reliability of one particular marker does not fulfill the goal of identification of genotype. Similarity coefficient on the basis of pooled study was ranged from 40 to 100%. Thus, mixed clustering pattern was much similar to SSR clustering pattern.

5.3 CONCLUSION

Physiological parameters of blackgram showed different results for the various salinity levels. Based on their performance, they were discriminated into tolerant, moderately tolerant and sensitive against salinity stress. Data on physiological parameters at the highest salinity level (ECe 8 dSm⁻¹) categorized the genotypes SKNU-03-03, SKNU-07-06, SKNU-06-03, SKNU-07-01 and IC-214520 as tolerant, IC-14520, IC-214844, IC-242677, SKNU-07-03, SKNU-2K-3, IC-204869, IC-61100, COBG-593, SKNU-03-04, SKNU-9911, TPU-94-2, TPU-4 and SKNU-9915 are moderately tolerant while GJU-1506, JAWAHAR URD-3, JAWAHAR URD-2 and GJU-1509 were sensitive to salinity. These genotype showed higher values for seedling length, and seedling dry weight. Looking to the vigour index I & II, genotypes JAWAHAR URD-3 and SKNU-9911 respectively showed higher values under maximum (ECe 8 dSm⁻¹) salt concentration.
Molecular markers RAPD, ISSR and SSR were reliable to distinguish blackgram genotypes for salinity tolerance. Molecular analysis for genetic diversity of different blackgram genotypes observed that under RAPD markers, genotypes SKNU-07-03 and SKNU-9911 observed maximum similarity which are categorized as salt tolerant genotypes and SKNU-07-01 was found as the most distinct among all the other genotypes. In ISSR markers, salinity tolerant genotypes IC-14520 and IC-214845 were showed maximum similarity. SSR markers and according to phylogenetic tree based on pooled data of molecular markers (RAPD, ISSR and SSR) categorized genotypes SKNU-06-03, SKNU-07-03, SKNU-9911, SKNU-2K-3, COBG-593 and TPU-4 as salinity tolerant which were showed as the most similar.