Chapter - V

Summary & Conclusion
SUMMARY AND CONCLUSION

The present study was aimed at a) Characterization of the chilli genotypes through different qualitative characters, b) Genetic variability for green chilli yield and yield components, c) Character inter­relationships and their direct and indirect effects on green fruit yield, d) Determination of genetic diversity of the genotypes through multivariate analysis and isozyme study e) Evaluation of the genotypes for tolerance to chilli leaf curl complex and determination of biochemical basis of tolerance f) Analysis of adaptability of the promising genotypes at different salinity levels through multilocational trial and g) Characterization of the selected genotypes based on morphological characters and molecular marker.

Thirteen distinct and species qualifying qualitative characters viz. growth habit, nodal anthocyanin pigmentation, leaf colour, leaf shape, flowering behaviour, corolla colour, corolla spot colour, anther colour, calyx margin, calyx angular constriction, fruit orientation, mature fruit colour and anthocyanin strip on fruit were recorded for all the 37 genotypes of chilli. It was found that most of the genotypes belonged to C. annuum. Only one genotype, CUCH-20 showed annular constriction in calyx and purple corolla colour closely resembling C. chinense. It was also presumed that this genotype might have developed through natural out crossing between C. annuum and C. chinense genotypes.

Thirty seven chilli genotypes were evaluated for seventeen growth, yield contributing characters and fruit yield for three consecutive years (2007-08, 2008-09 and 2009-10) following Randomized Block Design with 3 replications. All the 17 characters varied significantly among the genotypes indicating the presence of variability for the concerned characters. Mean sum of squares clearly suggested significant difference of the genotypes for all the characters. ‘Year x Genotype’ interaction was significant only for 3 characters viz., days to 50% germination, days to 50% flowering and fruit yield per plant indicating stable expression of
most of the characters over the years. In most of the characters, Phenotypic Coefficient of Variation (PCV) corresponded closely to Genotypic Coefficient of Variation (GCV) indicating stable expression of the characters under the particular environmental condition. Characters like number of primary branches per plant, fruit pedicel length and number of fruits per plant showed wide difference between PCV and GCV indicating significant genotype-environment interaction for the development of these characters. GCV for all the characters was low to moderate and least for 100-seed weight indicating comparatively less genetic variation among the genotypes for the concerned character. Moderate GCV coupled with high broad sense heritability and high genetic advance was registered in four characters namely fruit yield per plant, seeds per fruit, fruit girth and leaf width. Simple selection will be rewarding for improving these characters. Simple selection will also be effective for days to 50% flowering, fresh fruit weight, dry fruit weight, fruit pedicel length and number of fruits per plant which registered moderate GCV, coupled with high heritability and moderate genetic advance. Moderate to high heritability accompanied with low to moderately low genetic advance was recorded for plant height, plant canopy width, primary branches/plant, leaf length, fruit length and 100 seed weight. Improvement of these characters needs selection over several successive years.

These 37 chilli genotypes were also subjected to evaluationary trials for five physiological and fruit quality traits over three consecutive years following RBD with three replications. Mean sum of squares exhibited significant difference of the genotypes for all the characters. ‘Year X Genotype’ interaction was significant only for leaf phenol content while rest of the characters exhibited stable expression over the years. From the pooled analysis, it was clear that vitamin C content of the fruit showed a range of 62.06 to 137.28 mg/100g fresh weight suggesting ample scope for selection of high vitamin C containing chilli genotype. For all the 5 characters, PCV corresponded closely to GCV indicating less influence of environment for the expression of these characters. GCV for

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all the characters was moderate to moderately low indicating moderate genetic variation among the genotypes for the concerned characters. Moderate GCV coupled with high broad sense heritability and high genetic advance was registered for total carotenoid content, capsaicin content and total chlorophyll content of the fruits. Such combination of genetic variability parameters is attributable to additive gene action, hence, early generation selection would be helpful for improving these characters.

Among the Genotypic (G) and phenotypic (P) correlation coefficients, phenotypic correlation coefficients have been utilized to depict the character association. Fruit yield per plant was positively and significantly correlated with as many as 14 characters suggesting overwhelming importance of yield components for realizing fruit yield per plant. Highest and positive direct correlation with yield was recorded by fresh fruit weight followed by fruits per plant, dry fruit weight, 100 seed weight, vitamin C content, plant canopy width, total chlorophyll content in green mature fruit, fruit length, leaf length, total carotenoid content in ripe fruit, leaf phenol content, leaf width and plant height.

Fresh fruit weight recorded significant positive correlation with fruit length and leaf length. It suggested that selection for leaf length may increase fruit weight probably by enhancing the photosynthetic area. Significant and positive correlation between total chlorophyll content in green mature fruit and total carotenoid content in the red ripe fruit indicated the possibility of early selection based on chlorophyll content of fruit for high colour content at ripe stage. Significant and positive correlation between leaf width and days to 50% flowering indicated that early flowering genotypes basically possess narrow leaved character. Revelation of significant and positive correlation between fruits per plant and vitamin C content justified the possibility of simultaneous selection of higher fruiting and high vitamin C containing genotypes at the same time. 100 seed weight showed positive and significant correlation with fresh fruit weight, dry fruit weight and leaf width indicating widely perceived association between fruit and seed.
weight. Leaf phenol content registered positive and significant correlation with total carotenoid content of ripe fruit, total chlorophyll content in green mature fruit and number of fruits per plant.

The path coefficient analysis depicted that highest direct positive effect on fruit yield was exerted by fresh fruit weight followed by number of fruits per plant and leaf width. Fresh fruit weight also exerted high indirect effect on fruit yield per plant via leaf length, fruit length, and 100 seed weight. Indirect effect of fruit number per plant on fruit yield per plant via vitamin C content, leaf phenol content, total chlorophyll content in green mature fruit and total carotenoid content in red ripe fruit was also high and positive in direction. Ascorbic acid content, leaf phenol content, total chlorophyll content in green mature fruit and total carotenoid content in red ripe fruit was indirectly related to yield through number of fruits per plant. High direct negative effect on yield was observed from leaf phenol content followed by plant canopy width, days to 50% flowering and 100 seed weight.

Important selection indices as framed from the studies depicted that fresh fruit weight, fruits per plant, dry fruit weight, 100-seed weight and leaf width emerged as the most important characters influencing fruit yield. The other characters namely fruit length, vitamin C content of fruit, leaf phenol content, total chlorophyll content in green mature fruit and total carotenoid content in red ripe fruit too shared their importance as potent contributors towards fruit yield of chilli.

The genetic divergence of the 37 genotypes was analyzed employing 22 important characters pertaining to growth, fruit yield components, proximate compositions of leaf & fruit and fruit yield which grouped the genotypes into 7 clusters. It was found that the clustering pattern was, in general, not related to geographic origin of the genotypes. However, some genotypes having similar place of origin were grouped in the same cluster (UCH-20, CUCH-21, CUCH-22 and CUCH-23 from Assam & Nagaland in Cluster IV). Hence, implication of geographical origin on genetic diversity may not altogether be ignored.
Cluster IV was the largest containing 14 genotypes followed by cluster VII and II having 8 and 7 genotypes, respectively. The intra-cluster divergence was highly variable ranging between 30.25 in cluster II and 191.25 in cluster VII. Cluster II formed the most homogeneous group and cluster VII formed the most heterogeneous group. High heterogeneity was observed in cluster IV showing high intra-cluster divergence (184.46). The inter cluster D² value was maximum (248.09) between cluster V and VII. The minimum distance was between cluster I and VI (59.53).

Cluster VII had high mean values for maximum number of yield contributing characters as well as physiological and fruit quality characters viz., leaf width, fruit girth, number of fruits per plant, fresh fruit weight, dry fruit weight, total fruit yield per plant, total chlorophyll content in green mature fruit, total carotenoid content in red ripe fruit and leaf phenol content. Cluster VI recorded the highest mean value for fruit quality traits i.e. ascorbic acid content and capsaicin content but mean yield per plant of this cluster was very low.

In the peroxidase enzyme polymorphism study, peroxidase electrophoretic banding, zymotypes and zymogram grouped the genotypes into 6 clusters/zymotypes. Similarity matrix values suggested that the Zymotypes P₁ and P₄; P₄ and P₃; P₄ and P₅ were close to each other (Similarity index 0.857). The Zymotype P₆ was farthest from P₅ (Similarity index 0.286) followed by P₃ (Similarity index 0.333).

Number of clusters obtained by peroxidase zymogram analysis were almost same to multivariate analysis but the genotypes included in each cluster varied, which was an indicative of environmental influence for the expression of quantitative traits. It emerged that 73% of total genotypes (27 out of 37) shared similar groups under both multivariate analysis and isozyme polymorphism. The result amply suggests that these two methods of determining diversity are complementary to each other and should be carried out simultaneously to determine genetic diversity in a more fruitful manner.
Disease reaction of 37 genotypes were studied against chilli leaf curl complex by growing them for two consecutive years following RBD having 3 replications without application of any insecticides or acaricides. Coefficient of infection (CI) for leaf curl complex showed wide variation among the genotypes. Most of the genotypes were susceptible to this disease, registering high coefficient of infection. CI less than 20 was shown by only 8 genotypes which amply suggested the severity and wide spread occurrence of leaf curl disease. Range of CI was very wide from 8.75 in CUCH-4 to 93.75 in CUCH-23. According to disease reaction as depicted by CI, the genotypes were categorized into five groups. 'Resistant' group (CI <10) comprised of only 1 genotype viz. CUCH-4 and 'Moderately Resistant' group (CI 10-20) was having 8 genotypes. 'Highly Susceptible' group was the largest group comprising of 15 entries (CI >70). Rest 13 genotypes were placed into 'Moderately Susceptible' (CI 20-40)' and 'Susceptible (CI 40-70) groups.

Isozymes (Peroxidase & Polyphenol oxidase) activity was studied for all the 37 genotypes. Highest isozyme activity was expressed by the resistant genotype CUCH-4 (12.50 unit & 0.18 unit respectively /minute/g fresh tissue) and lowest by the highly susceptible genotype CUCH-23 (1.14 unit & 0.03 unit respectively /minute/g fresh tissue).

Clustering of the genotypes employing CI of leaf curl complex and plant defense related characters viz., phenol content, peroxidase activity and polyphenol oxidase activity grouped the genotypes into 3 clusters. Cluster I consisted of 5 genotypes, 1 'Resistant' (CUCH-4) and 4 'Moderately Resistant' (CUCH-29, CUCH-31, CUCH-34, CUCH-35). Rest members of 'Moderately Resistant' and all members of 'Moderately Susceptible' category were grouped into Cluster II. 'Susceptible' and 'Highly Susceptible' members formed Cluster III.

Correlation study revealed that CI of leaf curl disease was significantly and negatively correlated with leaf phenol content, peroxidase activity and poly-phenol oxidase activity. Significant positive correlation was observed for leaf phenol content with peroxidase and
poly-phenol oxidase activity. The correlation coefficient between peroxidase activity and poly-phenol oxidase activity was very high and significant. It emerged that higher the leaf phenol content, peroxidase activity and poly-phenol oxidase activity, the lower would be the leaf curl disease incidence.

Considering the clustering pattern of both the analysis, intra and inter cluster divergence, cluster mean values for different characters and reaction to leaf curl complex disease, 12 divergent genotypes were selected for further evaluation towards salinity stress of varied levels at three locations. Of these twelve genotypes one was resistant (CUCH-4), 8 were moderately resistant (CUCH-1, CUCH-5, CUCH-6, CUCH-7, CUCH-29, CUCH-31, CUCH-34, CUCH-35) and 3 were moderately susceptible (CUCH-11, CUCH-15, CUCH-16) to chilli leaf curl complex with comparatively lower values of coefficient of infection. These genotypes were grown in natural saline conditions having three levels of salinity viz., low (EC- 1.63 dS/m), medium (EC- 2.55 dS/m) and high (EC- 3.72 dS/m).

Mean values for 9 growth and yield related characters viz., plant height, primary branches per plant; days to 50% flowering, fruit length, fruit girth, fruits per plant, fruit weight, dry fruit weight and fruit yield per plant varied considerably with the salinity level. High salinity level drastically reduced the manifestation of all the characters including fruit yield in all the genotypes compared to low and medium salinity level. The reduction was more conspicuous for plant height and fruit yield per plant. Average fruit yield reduced by 20.62% when they were grown in the high salinity condition compared to low salinity condition. Mean sum of squares suggested significant difference of the genotypes for all the characters in all the three salinity levels. This finding endorses the selection of promising genotypes suitable for soil salinity condition. It was found that soil salinity increased the susceptibility of the genotypes to leaf curl disease which might have happened due to impaired nutrient uptake under saline situation. Out of these 12 genotypes, 7 genotypes (CUCH-1, CUCH-4, CUCH-6, CUCH-29, CUCH-31, CUCH-34 & CUCH-35)
showed comparatively low yield reduction (%) at high and medium salinity levels with respect to standard performance at very low salinity level in the instructional farm of KVK, Nimpith. CI for leaf curl disease for these 7 genotypes was low ranging from 13.13 to 31.88 at all the salinity levels. In these 7 genotypes, range of fruit yield reduction in high salinity level was 26.93% to 41.55% while that under medium salinity level was 13.33% to 26.34%. Lowest yield reduction at medium salinity level was recorded in the genotype CUCH-6 (13.33%) while that in the high salinity level was recorded in the genotype CUCH-35 (26.93%).

In the present study, the genotypes CUCH-6, CUCH-29 and CUCH-34 registered high mean fruit yield with unit regression coefficient close to 1.00 and very low $S^2_{di}$ values and could therefore, be considered as stable genotypes. Two genotypes viz., CUCH-1 and CUCH-31 showed high mean values for fruit yield but their $b_1$ values were more than 1.00 and $S^2_{di}$ values were low, thus suggesting that these two genotypes are fit to be adapted only under specific favourable environment (below average stability).

RAPD analysis of the genomic DNA isolated from the leaves of the 7 selected genotypes performing well in the multi-locational trial at different salinity levels was performed using 8 selected decamer primers, which generated 72 number of bands, of which 25 were monomorphic and 47 were polymorphic. Highest number of band output was observed under the primer OPA-12 (14) whereas OPB-11 resulted in lowest number of bands (6).

The dendrogram showed that the selected 7 genotypes were clustered into two major groups, one having only one genotype CUCH-1 and the other having rest 6 genotypes. The second group was again categorized into two sub groups, one having the genotype CUCH-29 and the rest five grouped into another. Of these five genotypes CUCH-4 and CUCH-6 formed a separate group and showed very close proximity. Three high yielding genotypes CUCH-31, CUCH-35 and CUCH-34
belonged to the same group of which CUCH-31 and CUCH-35 showed very close proximity.

Comparison of the RAPD grouping and multivariate clustering showed that genotypes CUCH-4 and CUCH-6 grouped in similar way in both the cases. Similar observation was also recorded for CUCH-31, CUCH-34 and CUCH-35. But unlike multivariate analysis, two genotypes (UCHU-1 and CUCH-29) showed complete distinctness at the genotypic level as expressed in RAPD analysis.

In isozyme analysis, it was observed that out of the seven genotypes taken for RAPD analysis, CUCH-1 belonged to zymotype P1; CUCH-4 and CUCH-6 to zymotype P2; CUCH-29 to zymotype P3 and rest three (CUCH-31, CUCH-34 & CUCH-35) to zymotype P4. This grouping pattern closely resembled the grouping pattern recorded in the present RAPD analysis. The study of genetic diversity following multivariate analysis based on quantitative characters, isozyme analysis based on biochemical (gene product) marker and RAPD analysis based on DNA marker amply suggests that these three methods of determining genetic diversity have their own limitations and may be referred to as complementary to each other. Hence, all the three methods should be carried out simultaneously to determine genetic diversity of chilli in a more fruitful manner.

CONCLUSION

It can be concluded from the present study that

➢ Considerable genetic diversity existed among the assembled genotypes. Thirty seven genotypes under study were grouped into seven clusters in D² analysis and six zymotypes in isozyme analysis.

➢ Most of the genotypes expressed 'susceptible' disease reaction to chilli leaf curl complex. Only 24.32% of the total genotypes exhibited some degree of resistance to leaf curl complex.
Correlation studies of chilli leaf curl complex and its biochemical basis revealed that a genotype expressed higher resistance to leaf-curl complex when it possesses higher phenol content, polyphenol oxidase activity and peroxidase activity.

Twelve better performing genotypes were put in multilocational salinity stress trial and only three genotypes viz. CUCH-6, CUCH-29 and CUCH-34 expressed stability under varying stress levels.

In case of dual stress tolerance for salinity and leaf curl complex, only the genotype CUCH-34 performed better as its ranking for disease reaction did not change with the level of soil salinity.

A combination of higher yield with high pungency (capsaicin content) was recorded in the genotype CUCH-34. So, this genotype may be considered for commercial cultivation of green chilli at the farmers’ field in the Sundarban region.

In multilocational salinity stress trial, CUCH-1 & CUCH-31 showed high mean values for fruit yield but their bi values were more than 1.00 and S^2di values were low suggesting that these genotypes are suited for specific favourable environment. Disease reaction of CUCH-31 was ‘moderately resistant’ under all salinity levels and it’s mean yield was 62% higher than CUCH-1. So, this genotype can be promoted for low salinity environment favourable for it’s higher yield.

Genotype CUCH-31 scored highest amount of carotenoid content in dry red fruit in combination with higher yield potential. Fruit length of this genotype is considerably higher, making it a promising line for dry chilli production.

Genotype CUCH-4, resistant to leaf-curl complex with higher yield potential (132.8 g/plant) and showing uniformity in disease reaction (MR) at all the salinity conditions, can be used as parental material in resistance breeding programme.
FUTURE SCOPE OF RESEARCH

❖ The genotype CUCH-4, possessing resistance towards leaf-curl complex may be forwarded to the state/national varietal release committee for further scoring to judge its superiority and seek opinion to release it as a variety.

❖ The inheritance pattern of resistance towards chilli leaf curl complex in some of the selected genotypes may be determined at advanced molecular level so as to assemble the requisite genes for breeding of chilli genotypes as per the desired nomenclature.

❖ Chilli accessions screened for tolerance to soil salinity viz. CUCH-6, CUCH-29 and CUCH-34 may also be subjected to genetic evaluation at molecular level to have a better understanding of its inheritance pattern and utilize the same in advance breeding programmes.

❖ Diverse chilli genotypes may be added on to the already existing gene pool to enhance the genetic base in getting a wide array of parental lines for future breeding programme.