

Screening of Brinjal Genotypes of North East India against Bacterial Wilt Using SSR Markers

Thesis

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Master of Science (Horticulture)

In
Vegetable Science
by

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U-18-AR-01-005-M-H-003



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December 2020

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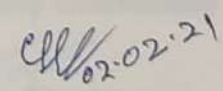
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CERTIFICATE – II

This is to certify that the thesis entitled “**Screening of Brinjal Genotypes of North East India against Bacterial Wilt Using SSR Markers**” submitted by **Ms. Tasso Annu** [Registration No. **U-18-AR-01-005-M-H-003**] submitted to the Central Agricultural University, Imphal-795004 (Manipur) in partial fulfilment of the requirements for the award of the degree of **Master of Science (Horticulture)** in the subject of **Vegetable Science** has been approved by the Student's Advisory Committee after oral examination jointly with Dean's Nominee.

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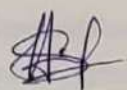
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I hereby declare that the thesis entitled **"Screening of Brinjal Genotypes of North East India against Bacterial Wilt Using SSR Markers"** is an authentic record of the work done by me and that no part thereof has been presented for the award of any other degree, diploma, associateship, fellowship or any other similar title.

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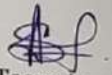
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LIST OF SYMBOLS/ABBREVIATIONS

CD	Critical difference
CV	Coefficient of variation
°B	°Brix
°C	°Celcius
CTAB	Hexadecyl trimethyl-ammonium bromide
DNA	Deoxyribonucleic acid
µg	Microgram
µl	Microlitre
/	Per
%	Percent
cm	Centimeter (s)
<i>et al.</i> ,	Co-workers
EDTA	Ethylene diamine tetra acetic acid
g	gram (s)
ha	Hectare
K ₂ O	Potassium oxide
kg	Kilogram
m	Metre (s)
M	Molar
mL	Milliliter
MSL	Mean sea level
MT	Metric tonne (s)
N	Nitrogen
NaOH	Sodium hydroxide
nm	Nanometer

OD	Optical density
P ₂ O ₅	Phosphorus pentoxide
PVP	Polyvinyl pyrrolidone
QTL	Quantitative trait loci
RBD	Randomized Block Design
Sl.No.	Serial number
t	Tonne (s)
TAE	Tris-acetate-EDTA
<i>viz.</i> ,	Namely
V	Volt



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ABSTRACT

An experiment entitled "Screening of Brinjal Genotypes of North East India against Bacterial Wilt Using SSR Markers" was conducted at polyhouse of Vegetable Research Farm, Deptt. of Vegetable Science, College of Horticulture and Forestry, CAU, Pasighat, Arunachal Pradesh during the year 2019-20 with 22 brinjal genotypes in Randomised Block Design with three replications.

Genetic variability and correlation were studied and the characters like fruit weight (g), number of fruits per plant, fruit girth (cm), fruit yield per plant (kg) had high PCV and GCV whereas, heritability was observed high for all the characters except for days to first harvesting (48.40%). Genetic advance was highest for fruit weight (229.06%). Correlation study indicated that fruit yield per plant was positively and significantly correlated with fruit weight, leaf width and number of fruits. Maximum inter cluster distance was observed between cluster III and V and indicated that the genotypes within these were highly divergent.

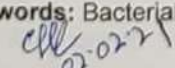
For screening of bacterial wilt severity, days to first incidence of bacterial wilt symptoms, plant disease incidence at 1-15, 15-25, 25-30, 30-50, 50-60 DAI, and disease severity index were recorded. Disease severity was found lowest in CHFB-33, followed by CHFB-29 and CHFB-22. On the basis of screening in field condition, the genotypes CHFB-22, CHFB-29, CHFB-33 were found resistant, CHFB-21, CHFB-23, CHFB-24, CHFB-26, CHFB-28, CHFB-30, CHFB-31, CHFB-34, CHFB-35 were found moderately resistant, CHFB-20, CHFB-25, CHFB-18, CHFB-19 moderately susceptible, CHFB-37, CHFB-27, CHFB-32 susceptible and CHFB-36 highly susceptible against bacterial wilt of brinjal.

DNA extraction was done by CTAB method and PCR using 20 SSR markers. Primer emb01J19 showed highest polymorphic information content, diversity index and matrix index. In the study of molecular diversity, genotypes were grouped broadly into two major clusters I and II, where cluster I is further divided into sub clusters IA and IB using SSR markers. The sub cluster IA again divided into two sub clusters, IA₁ and IA₂ on molecular basis.

All the genotypes grouped under above clusters considered in two main clusters on the basis of its diversity, resistant and susceptibility. The genotypes Arka Nidhi and Arka Keshav were recorded to be highly resistant which falls under sub cluster IA. The genotypes CHFB-22, CHFB-26, CHFB-29, CHFB-21, CHFB-24, CHFB-23, CHFB-31, CHFB-26, CHFB-28, CHFB-30, CHFB-33, CHFB-34, CHFB-35, CHFB-19, CHFB-18, CHFB-37 CHFB-20, CHFB-25, CHFB-36 observed resistant to moderately susceptible under major cluster I, while, CHFB-27 and CHFB-32 grouped under major cluster II showed susceptibility through SSR markers. Although genotype CHFB-36 observed most susceptible in field condition, it was grouped under major cluster I in molecular analysis due to the similar genetic makeup of the genotype with other genotypes of major cluster I.

It is concluded that genotypes CHFB-22 and CHFB-29 found nearer to resistant check varieties Arka Nidhi and Arka Keshav under major cluster I and also CHFB-33 appeared separately in major cluster I sub cluster IA- IA₁ grouped under resistant and these three lines can be utilized for improvement through breeding.

Keywords: Bacterial wilt, brinjal, diversity, SSR markers, yield.


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Chapter-1

Introduction

Brinjal is considered to be the king of vegetables. It is also known as egg plant and is the second most important solanaceous fruit crop after tomato in the genus *Solanum*. Brinjal (*Solanum melongena* L.) is an important vegetable grown in India and other parts of the world (Khapte *et al.*, 2012; Frary *et al.*, 2007). It belongs to the family Solanaceae and has a chromosome no. $2n=24$. It is usually self pollinated, but the extent of cross pollination has been reported to be as high as 48% and therefore it is classified as often cross pollinated crop.

India is one of the largest brinjal producing countries in the world covering an area of 0.73 million hectares with a production of 12.80 million tones and a productivity of 17.50 t/ha. (Anonymous, 2021).

Brinjal has been divided into three main types, *S. melongena* var. *esculentum*, var. *serpentium* and var. *depressum*. There are also many wild species of eggplant that carry many important genes. Wild eggplant species show resistance to important diseases and pests that affect commercial eggplant production (Chen and Li, 2000). Among the wild relatives of eggplant which can be exploited to increase genetic variability, *S. torvum* has been reported to be useful for introgression of disease resistance (Collonnier *et al.*, 2001; Daunay *et al.*, 1991). *S. torvum* has been identified to carry traits of resistance to most serious diseases of eggplant, particularly bacterial and fungal wilts. *S. aethiopicum* is less susceptible to many pathogens, including fungi (*Fusarium* spp.), bacteria (*Ralstonia solanacearum*) (Toppino *et al.*, 2008; Rizza *et al.*, 2002) and root-knot nematodes (*Meloidogyne* spp.). *Solanum* is one of the largest genera of vascular plants having more than 1,500 described species (Li, 2000).

Brinjal grows 60-120 cm tall either erect or semi-erect. The plant produces numerous branches and has fibrous or lignified roots with deep growing tap root system. The leaves are large, simple, lobed and alternate on the stems. The flowers are large, violet or white coloured and are born solitarily or in cluster. Brinjal fruits are smooth, shiny having wide variability of colours like black, purple, green, violet and green with white variegation. The shape of the fruit varies from ovoid, oblong, or long cylindrical. Though brinjal is self pollinated crop, heteromorphic flower structure *i.e.*, heterostyly enables high degree of cross pollination. Different types of flower are: (a) long styled (b) medium styled (c) short styled and (d) pseudo short styled.

Brinjal is a good source of nutrients, minerals, antioxidants, vitamins, dietary fibre and body building factors and proteins (Matsubara *et al.*, 2005; Obho *et al.*, 2005). It is low in calories and fats and mostly contains water. Fruits are widely used in various culinary preparations. It has got much potential as raw material in pickle making and dehydration industries. Fruits are excellent remedy for those suffering from liver troubles and high blood cholesterol (Singh and Kalda, 2001). The de-cholesterolizing action is attributed to the presence of poly-unsaturated fatty acids (linoleic and lenolenic) in fruits. White brinjals are said to be a remedy for diabetic patients (Choudhury, 1976).

Many species of plant are being infected by bacterial wilt especially solanaceae crops (Liu *et al.*, 2016). Among solanaceous vegetables, brinjal is more susceptible with yield losses ranging from 65 to 70% (Das and Chattopadhyay, 1953). The recent studies reported the yield losses for about 4.24 to 86.14 per cent (Sabita *et al.*, 2000). Also among all the diseases of brinjal, one of the most devastating diseases is bacterial wilt. Bacterial wilt caused by *Ralstonia solanacearum* (Yabuuchi *et al.*, 1995) previously known as *Pseudomonas solanacearum* is widely distributed throughout tropical, sub-tropical and temperate regions of the world (Hayward, 1991). It is a soil-borne disease which causes damage in all growth stages of brinjal. Based on their host range, the bacteria have been classified into five races (He *et al.*, 1983; Buddenhagen *et al.*, 1962; Coutinho, 2005) and six biovars (Buddenhagen *et al.*, 1962). The bacterium can infect over 450 hosts and cause loss in yield up to 90% in solanaceous vegetables (Elphinstone, 2005; Wicker *et al.*, 2007; Yuliar *et al.*, 2015).

Ralstonia solanacearum is an aerobic, gram negative, non-spore forming, rod-shaped bacterium. It can spread rapidly at 25 to 35°C and is favoured by high humidity conditions (Ramesh *et al.*, 2009; Lebeau *et al.*, 2011). The bacteria can survive for long periods in the soil which ranges from one to ten years without a host plant.

The bacterium generally enters the plant through roots via wounds or the natural openings from which secondary roots emerge (Hayward, 1991). Initially the symptoms can be seen as drooping of leaves; it then progressively invades the stem vascular tissues and blocks the vessels by bacteria finally leading to partial or complete wilting of plant (Sarkar and Chaudhuri, 2016). Discoloration of vascular tissues can also be found in the wilted plant. When the cut ends of the stem/root is kept in a clear container of water in an undisturbed manner for few minutes, a stream of milky white bacterial ooze can be seen coming out. This is a simple diagnostic method to identify bacterial wilt in field condition.

Since it has a wide host range and the disease is widely distributed, it becomes quite difficult to manage or control this disease with chemical, biological and cultural practices (Grimault *et al.*, 1993). Use of resistant varieties is one effective approach against the problem. Growing resistant brinjal variety is eco-friendly, durable and an effective control/ management of the disease. But it has been observed that most of the varieties which are available in the market have moderate or low resistance to bacterial wilt. Therefore, there is a need to screen out genotypes which are resistant to bacterial wilt. In the absence of effective chemicals and bactericides for managing this disease, emphasis is laid on developing eggplant (brinjal) cultivars with resistance to *Ralstonia solanacearum*.

Since the last five decades, molecular markers have been playing an important role in genetic improvement, disease resistance, gene tagging, germplasm characterization mapping, QTL analysis, paternity analysis, finger printing and utilization in a wide range of horticultural crops. Simple sequence repeats (SSRs) or microsatellite markers are ideal DNA markers due to its hypervariability, co-dominant nature, multiallelic, reproducibility, genomic abundance and coverage (Kalia *et al.*, 2010).

PCR amplifies and detect these SSR polymorphisms at individual loci using locus-specific flanking area primers where the sequence is identified. The best approaches would therefore be to search for a resilient source and to incorporate genes into commercial cultivars.

In the case of eggplant, SSR markers have been developed recently and mainly used for the assessment of genetic diversity and genome similarity in the related species (Nunome *et al.*, 2003; Nunome *et al.*, 2009; Demir *et al.*, 2010; Fukuoka *et al.*, 2012; Chourey *et al.*, 2017), genetic diversity of eggplant and wild species (Caguiat and Hautea, 2014); parent specific SSRs (Liu *et al.*, 2016), protection of eggplant germplasm (Prohens *et al.*, 2011) and hybrid purity testing (Kumar *et al.*, 2014).

Polymorphisms between cultivars and breeding lines should be identified for molecular breeding. However, a low frequency of polymorphism among cultivars and intraspecific lines has been reported in solanaceous plants.

Studies pertaining to use of SSR markers for the identification of diseases such as bacterial wilt in eggplants are very meagre, the literature survey shows that SSRs in eggplant have used either molecular diversity or hybrid purity analysis. The aim of the present study was therefore to screen elite eggplants

genotypes (advance breeding line) in field conditions for bacterial wilt and to identify bacterial wilt resistant brinjal genotypes.

At present, very few brinjal varieties/hybrids from public and private sector show moderate or low resistance against bacterial wilt while on real ground, the bacterial wilt affect yield loss up to 80% (Sabita *et al.*, 2000). Hence selecting molecular markers which are tightly linked to resistance genes will help in identifying brinjal genotypes resistant against bacterial wilt and it can further be used in the breeding program. Considering the above criteria for study, the topic “**Screening of Brinjal Genotypes of North East India against Bacterial Wilt Using SSR Markers**” will be taken up with the following objectives in mind:

1. Screening of bacterial wilt severity in brinjal genotypes.
2. Assessment of bacterial wilt resistant brinjal genotypes based on molecular analysis.

Chapter-2

Review of Literature

In this chapter, the literature pertaining to various aspects of the study are reviewed in brief under the following heads:

2.1 Genetic variability

2.2 Heritability and genetic advance

2.3 Correlation studies

2.4 Path coefficient analysis

2.5 Genetic divergence

2.6 Morphological and biochemical features of *Ralstonia solanacearum*

2.7 Genetics and resistance breeding for Bacterial Wilt

2.8 Markers

2.1 Genetic variability

The improvement in any crop is proportional to the magnitude of its genetic variability present in the germplasm. Greater the variability in a population, greater is the chance for effective selection for desirable types (Vavilov, 1951). The variability observed in any population could be due to two factors i.e. genetic and environmental, which was discussed in the early part of last century by Johannsen (1909) who attributed the variation in a segregating population to both heritable and non-heritable factors and variation within pure line to only environmental factors. A wide range of variability is available in brinjal genotypes which provide great scope for improving fruit yield through systematic breeding. To initiate any breeding work, it is necessary to assess the genetic variability present in the indigenous genotypes for yield and its components. Parameter of genotypic and phenotypic coefficient of variations is useful in detecting the amount of variability present in the germplasm (Datta and Das, 2013). A brief review on extend of variability for different traits are stated below:

Hassan *et al.* (2015) studied twenty two different eggplant for various agro-morphological traits and observed average to high variance for most of the parameters studied *viz.*, fruit weight, days to flowering and plant height. The fruit shape and colour observed in the germplasm accession was diverse ranging from round type

to elongated and dark purple to white. However, predominant shape was round (40.9%) type and purple (54%).

Madhavi *et al.* (2015) evaluated twenty one diverse genotypes of brinjal and revealed high phenotypic and genotypic coefficients of variation (PCV and GCV) for number of fruits per plant, average fruit weight, fruit yield per plant, fruits per cluster, flowers per cluster and fruit diameter.

Mili *et al.* (2014) studied genetic variability in thirty six genotypes of brinjal and observed significant differences for all the characters under study. High genetic coefficient of variation was observed for single fruit weight, fruit diameter, fruits per plant, fruit yield per plant and fruit length.

High PCV and GCV in number of branches per plant, fruit length, fruit breadth, number of fruits per plant, average fruit weight and fruit yield per plant was also observed by Kumar *et al.* (2014).

Prabakaran *et al.* (2013) reported high estimate of phenotypic coefficient of variation and genotypic coefficient of variation occurred for number of primary branches per plant, internodal length, fruit length, number of fruit per plant, average fruit weight, fruit yield per plant, total number of harvests recorded high (>25%) PCV and GCV for fruits per plant, average fruit weight, fruit length, Singh *et al.* (2013) fruit diameter, primary branches per plant, whereas, yield per plant, plant spread, plant height, days to first flowering, days to first fruit set and days to first harvest showed moderate PCV (10-25%).

Ansari (2010) at Raipur studied the association analysis in total fruit yield per plant showed positive and significant correlation with marketable fruit yield per plant, number of primary branches per plant, number of fruits per picking and total number of fruit per plant in both the seasons; number of fruits per cluster in rainy season only, whereas, negative significant correlation with days to first picking, days to first flowering, days to 50% flowering, days to first fruiting in both seasons.

Ram *et al.* (2007) at Kalyanpur observed high genotypic and phenotypic coefficient of variation for yield per plant, number of fruits per plant and plant spread in parents. High heritability coupled with high genetic advance indicating additive gene action was exhibited by characters, plant height, days to marketable maturity, plant spread, days to flowering, fruit yield per plant, fruit weight and number of branches per plant in F1s, F2s and parent populations.

Patel (2007) observed high estimates of PCV coupled with GCV in fruit weight, fruit yield per plant, fruit length, number of flowers per cluster and fruit stalk

length. High heritability coupled with high genetic advance as per cent of mean were observed for days to 50% flowering, fruit yield per plant, fruit weight, number of flowers per cluster, plant height, fruit length, fruit girth and fruit stalk length.

Mohanty and Prusti (2002) evaluated 15 cultivars of brinjal for 6 economic characters and found PCV higher than GCV for all the traits. High heritability accompanied by moderate to high GCV and genetic gain for yield, average fruit weight, number of fruit and branches per plant. Plant height and days to first harvest manifested high heritability coupled with low GCV and genetic gain.

2.2 Heritability and genetic advance

Sadarunnisa *et al.* (2015) studied genetic divergence among 50 genotypes of brinjal and revealed that the characters like average fruit weight, days to last harvest and bacterial wilt incidence contributed maximum to genetic divergence and hence played a major role in improvement of brinjal.

Yadav *et al.* (2014) observed high heritability coupled with high genetic advance in brinjal for average fruit weight, number of fruits per plant and fruit yield per plant.

Prabakaran *et al.* (2013) reported high estimate of phenotypic coefficient of variation and genotypic coefficient of variation occurred for number of primary branches per plant, internodal length, fruit length, number of fruit per plant, average fruit weight, fruit yield per plant, total number of harvests.

2.3 Correlation Studies

Ansari (2010) at Raipur studied the association analysis in total fruit yield per plant showed positive and significant correlation with marketable fruit yield per plant, number of primary branches per plant, number of fruits per picking and total number of fruit per plant in both the seasons; number of fruits per cluster in rainy season only, whereas, negative significant correlation with days to first picking, days to first flowering, days to 50% flowering, days to first fruiting in both seasons.

2.4 Path coefficient analysis

Ansari (2010) at Raipur studied the path analysis and revealed that in summer, total fruit yield per plant as dependent trait indicated that marketable fruit yield per plant showed highest positive direct effect, followed by days to first fruiting, average fruit weight, stalk length and number of primary branches per plant had positive direct effect. While, in rainy days to 50% flowering showed highest positive direct effect

followed by number of flower per inflorescence, number of fruits/plant, number of calyx, number of branches/plant, average fruit weight, plant height and days to first picking.

2.5 Genetic divergence

Sadarunnisa *et al.* (2015) studied genetic divergence among 50 genotypes of brinjal and revealed that the characters like average fruit weight, days to last harvest and bacterial wilt incidence contributed maximum to genetic divergence and hence played a major role in improvement of brinjal.

Patel (2007) at Raipur assessed sixty three genotypes of brinjal for genetic divergence. The maximum intra-cluster distance was obtained in cluster IV while, inter-cluster distance was highest between cluster II and III. Cluster I exhibited high cluster mean value for plant height and fruit yield per plant whereas, cluster III showed high cluster mean value for fruit girth, days to 50% flowering and fruit weight. The maximum contributions towards total genetic divergence were obtained from fruit yield per plant.

2.6 Morphological and biochemical features of *Ralstonia solanacearum*

Molecular detection methods are also there and are considered to be highly specific, sensitive and rapid. Strains of *Ralstonia solanacearum* isolated from bacterial wilt affected plants of brinjal, chilli and tomato were compared based on the utilization of carbohydrates, hypersensitivity reaction on capsicum leaves and RAPD analysis and based on the results the strains have been grouped into various biovars and races (James *et al.*, 2003).

Ralstonia solanacearum is widely distributed and found in tropical, subtropical, and some temperate regions of the world. The species as a whole has a very broad host range and infects hundreds of species in many plant families. The majority of hosts are dicots with the major exception being bananas and plantains. Most economically important host plants are found in the Solanaceae or nightshade family (Stevenson *et al.*, 2001).

Poussier *et al.* (2000) assessed the genetic diversity among the strains in a worldwide collection of *Ralstonia solanacearum*, the causal agent of bacterial wilt using AFLP markers which permitted very fine discrimination between different isolates.

Ralstonia solanacearum (Smith) (Yabuuchi *et al.*, 1995) (Formerly called *Pseudomonas solanacearum*), is a soil-borne bacterial pathogen and a major limiting factor in the production of many crop plants around the world. This organism is the causal agent of brown rot of potato, bacterial wilt or southern wilt of tomato, tobacco, eggplant and some ornamentals, and Moko disease of banana (Agrios, 1997).

R. solanacearum is a highly variable species encompassing six biovars based on biochemical properties (He *et al.*, 1983) and five races based on host range (Buddenhagen *et al.*, 1962; Pegg and Moffett, 1971). These host ranges and distributions have been changing in recent years (Denny, 2000).

2.6.1 Epidemiology of *Ralstonia solanacearum*

The pathogen infects roots through wounds and colonizes the vascular tissue causing plugging of the xylem and leaf wilting. There is often an association between nematode infection and bacterial wilt, where the nematodes create wounds in the root tissue to allow an entry point for the bacterium to infect the plant. The bacterium survives in the soil and can maintain infectious populations over several years. Incubation period of the bacterium is longer in resistant and moderately susceptible accessions (Hussain, *et al.*, 2005). Alternative weed hosts may also play a role in survival and over seasoning. Temperature is a major determinant in the distribution of this pathogen, which is widespread in tropical, sub-tropical and warm temperate regions where the mean soil temperature is greater than 15°C. Wet soil increases the incidence of disease and water movement contributes to the dissemination of inoculum with water movement. The incidence and rate of wilting therefore increases with high temperature and soil moisture. Continuous cropping of susceptible plants will also favour infection. Bacterial wilt of potatoes is more prevalent in slightly acid to acid soils, as are many soil-borne diseases. The pathogen is disseminated by contaminated farming equipment, on tyres and footwear in soil, drainage water carrying inoculum through the soil, infected seed.

2.7 Genetics and resistance breeding of bacterial wilt

2.7.1 Genetics of Bacterial wilt

Kumar *et al.* (2019) reported that among 30 genotypes of eggplant, IIHR-500A showed maximum resistance with no wilting symptom while eight genotypes namely IIHR-127, IIHR-575, IIHR-584, IIHR-667A, IIHR-766A, IIHR-817, IIHR-874, IIHR-882 showed resistant reaction with good yield potential. Three genotypes viz., IIHR 438-2, IIHR-618, IIHR-766 showed moderately resistant reaction and another

three IIHR-576, IIHR- 583 and IIHR-798 moderately susceptible reaction. The genotypes IIHR-574, IIHR-692, IIHR-793 and IIHR-857 were susceptible to bacterial wilt and 11 genotypes namely IIHR-228, IIHR- 534, IIHR-569, IIHR-571, IIHR-632, IIHR-711, IIHR-694, IIHR-812, IIHR-813, IIHR-880, IIHR-881 were highly susceptible.

Ajjappalavara *et al.* (2008) conducted an experiment in which it showed that the segregation of population to the inheritance of bacterial wilt resistance was 3 (non-wilted):1 (wilted) ratio and suggested single gene inheritance for bacterial wilt resistance.

Gopalakrishnan *et al.* (2005) demonstrated, inheritance study of susceptibility to bacterial wilt was dominant over resistance in brinjal.

Zhu *et al.* (2004) revealed that the resistance of bacterial wilt was controlled by single dominant gene.

Feng *et al.* (2003) concluded the inheritance of bacterial wilt resistance as additive-dominant model. Additive, dominant and recessive effects played major role in the genetics of bacterial wilt resistance, with additive effects being the dominant factor.

Pathania *et al.* (1996) evaluated fourteen varieties of brinjal, including the susceptible checks Pusa Purple Long, at Palampur and recorded highly significant differences amongst the varieties for plant survival. The highly resistant varieties Arka Neelkanth and Arka Keshav gave poor yields. Thus, they concluded that the long fruited types Arka Nidhi and Pusa Purple Cluster appear to be promising for use in varietal improvement or for cultivation in *Ralstonia solanacearum* prone areas of the western Himalayas. According to them round fruited type H-8 and oblong fruited type SM 6-7 also offer promise.

Mukherjee and Mukopadhyay (1982) categorised Pusa Purple Long cultivar as the most susceptible and Pusa Purple Cluster the most resistant, while Black Beauty as susceptible, Pusa Kranti moderately resistant and Improved Muktakeshi resistant to bacterial wilt.

Gopimony and George (1979) reported that in various district and agricultural farms in Kerala, the percentage wilt incidence in certain improved varieties like, Arka Kusumakar and Banaras Giant was as high as 100 per cent whereas, in local varieties it varied from 6 to 20 per cent.

The study of inheritance pattern of resistance to biotic stresses is essential for developing resistant hybrid/variety. The bacterial wilt inheritance is race,

biovar, strain, genotype and environment specific and it is genetically determined. According to Akiba (1972), the resistance to bacterial wilt in '*Nihan nassu*' cultivar was due to the presence of single dominant

2.7.1 Resistance breeding of bacterial wilt:

Namisy *et al.* (2019) conducted an experiment in which the objective was to screen seven accessions of cultivated eggplant (*Solanum melongena* L.) and 40 accessions from 12 wild relatives for resistance to two virulent *R. solanacearum* strains (Pss97 and Pss2016; phylotype I, race 1, biovar 3). The resistant or moderately resistant accessions were further evaluated with Pss97 in a second trial under high temperatures (and also with Pss2016 for *S. anguivi* accession VI050346). The resistant control EG203 was resistant to Pss97, but only moderately resistant to Pss2016. One accession of *S. sisymbriifolium* (SIS1) and two accessions of *S. torvum* (TOR2 and TOR3) were resistant or moderately resistant to Pss97 in both trials. *Solanum anguivi* VI050346, *S. incanum* accession MM577, and *S. sisymbriifolium* (SIS1 and SIS2) were resistant to Pss2016 in the first trial. However, *S. anguivi* VI050346 was susceptible in the second trial. These results are important for breeding resistant rootstocks and cultivars that can be used to manage this endemic disease.

Kumar *et al.* (2014) evaluated nine accessions of brinjal in initial evaluation trial and eight in advanced varietal trial (AVT) for bacterial wilt resistance. In IET Arka Nidhi, was found resistant to bacterial wilt. BEBWRES-3 was moderately resistant whereas other entries were found to be moderately susceptible/susceptible/highly susceptible. In AVT two entries BEBWRES-05 and Arka Nidhi exhibited resistant reaction. BEBWRES- 2, BEBWRES- 4 and SM 6-6 (C) were moderately resistant to bacterial wilt whereas, other cultivars were susceptible and highly susceptible during the year. Arka Nidhi was most resistant with 7 per cent wilt incidence. The accession BEBWRES-05 showed 19 per cent while BEBWRES 2 and SM6-6 showed less than 40 per cent wilt incidence.

Gopalakrinshnan and Reddy (2014) screened fifty genotypes of brinjal for resistance to bacterial wilt, four genotypes, viz., Arka Keshav, Surya, Arka Neelkanth and Arka Nidhi were found to be resistant, while 17 accessions were found to be susceptible and 29 accessions were found to be highly susceptible to bacterial wilt. In the susceptible check, Arka Shirish 90.67 per cent bacterial wilt incidence was recorded.

Mondal *et al.* (2011) evaluated six bacterial wilt resistant lines, they observed that BCB-35 gave highest yield but showed highly susceptible reaction to

bacterial wilt whereas, the lines BCB-14 and BCB-30 showed certain level of resistant reaction to bacterial wilt with high yield potential.

A study was conducted during 2010-2012 by Bainsla *et al.* (2016) to understand the genetic behaviour of bacterial wilt resistance in brinjal “CARI-B-1” (R) × “Pusa Purple Long” (S) in hot humid tropical climate of Andaman Islands. For this, the population from single F1 fruit was advanced to F2 and F3 and recorded the reaction of segregating population in the sick plots. The results revealed that there is preponderance of recessive gene family wherein more than one gene acts in additive mode. Another cross between *S. torvum* (R) × Diglipur local collection (S) also showed the recessive gene action for resistance as observed in F2 generation. Besides, the study also advocated that mechanisms of resistance, escape with early resistance and progressive escape have been found to be operating in individuals of segregating population.

Bi-hao *et al.* (2009) reported that the inheritance of bacterial wilt resistance in eggplant was controlled by a single dominant gene showing Mendelian inheritance model

Clain *et al.* (2004) reported that, *Solanum torvum* Sw. a wild species closely related to eggplant (*Solanum melongena* L.) has been identified as a potential source of resistance to bacterial wilt disease for cultivated susceptible Solanaceae species. It has been found that *Solanum torvum* can be considered as resistant to the race 1 biovar 1 strain and tolerant to the race 1 biovar 3 and race 3 biovar 2 strains of *Ralstonia solanacearum*.

Collonnier *et al.* (2001) conducted an experiment on which *Solanum aethiopicum* is reported to carry resistance to bacterial wilt disease caused by *Ralstonia solanacearum*, which is one of the most important diseases of eggplant (*Solanum melongena* L.). Somatic hybrids between *Solanum melongena* cv. Dourga and two groups of *Solanum aethiopicum* produced by electrical fusion of mesophyll protoplasts were found tolerant to *Ralstonia solanacearum* as about 50% of plants wilted. All somatic hybrids tested were as tolerant as the wild species.

Chaudhary and Sharma (2000) conducted an experiment on which nine genotypes of brinjal [aubergines] (*Solanum melongena*) viz., Arka Kesav, Arka Neelkanth, Hisar Shyamal, Pusa Purple Cluster, Pusa Purple Long, SM 6-6, SM 6-7, Arka Nidhi and Punjab Barsati were evaluated during 1996-98 for the incidence of bacterial wilt (caused by *Ralstonia solanacearum*) and attack of fruit and shoot borer (*Leucinodes orbonalis*) in the mid-hills of Himachal Pradesh, India. The genotypes Arka

Kesav, Arka Neelkanth, Arka Nidhi and SM 6-6 were resistant to bacterial wilt whereas fruit borer incidence in these varieties varied from 2.88 (Arka Kesav) to 5.64 (SM 6-6) percent. The cultivars also possessed good agronomic traits.

Chaudhary and Sharma (1999) conducted an experiment on which the bacterial wilt (*Ralstonia solanacearum*) resistant aubergine varieties Arka Kasev and Arka Neelkanth were crossed with susceptible variety Pusa Purple Long to study inheritance of disease resistance. Segregation of F₂ progenies of both crosses indicated the presence of single dominant gene controlling resistance to bacterial wilt. It is recommended to incorporate resistance into the susceptible cultivars through back cross pedigree method.

Solanum commersonii, a wild tuber-bearing species native to Uruguay with high potential for use in potato breeding programs has been found to carry resistance against the bacterial pathogen *Ralstonia solanacearum*, the causative agent of bacterial wilt (Laferriere *et al.*, 1999).

Singh and Gopalakrishnan (1998) evaluated 14 elite brinjal genotypes in the wilt sick soil of the warm humid tropical climate of Vellanikkara, Kerala, India and reported that varieties Surya, Swetha, Annapurna, BB7, BB13-1, BB44 and Arka Keshav were resistant to bacterial wilt.

Since agro-chemicals are not effective and sanitary cropping systems difficult to apply, control strategies of disease resistance have so far mainly consisted in plant breeding. Although screenings of eggplant accessions were conducted to find sources of resistance and despite the fact that some resistant varieties have been developed (Rao *et al.*, 1976; Goth *et al.* 1991; Peter *et al.*, 1993), the level of resistance has become insufficient in hot planting season or poorly drained fields (Ano *et al.*, 1991). Traits of resistance against bacterial wilt have been identified in different wild relatives of eggplant, such as *Solanum torvum*, *S. sisymbirifolium* (Dauny *et al.*, 1991).

2.8 Markers

2.8.1 Morphological markers

Pandiyaraj *et al.* (2019) conducted an experiment which consisted of seven germplasm viz., CARI-1, Surya, WCGR, IIHR-3, S-75, Rampur Local and IIHR-586. The experiments were conducted in bacterial wilt sick plot with artificial inoculation of *R. solanacearum* culture. The highest variance was observed for fruit weight and lowest variance was observed on fruit yield per plant. The germplasm Surya was

recorded as a better performed germplasm in respect to fruit yield per plant, number of fruits per plant, days to first flowering, number of primary branches per plant and percent disease incidence (bacterial wilt). The other germplasm CARI–1 was found to be immune to bacterial wilt disease with better yield performance.

Kamra and Kathuria (2016) elaborated that morphological markers are those traits that are scored visually, or morphological markers are those genetic markers whose inheritance can be followed with the naked eye. They masks the effect of linked minor gene, making it nearly impossible to identify desirable linkages for select and are limited in number, influenced by environment and also specific stage of the analysis.

2.8.2 Molecular markers

2.8.2.1 DNA based markers

DNA based assays have revolutionized and modernized our ability to characterize genetic variation. The first advantage of molecular techniques is their capacity to detect genetic diversity at a higher level of resolution; furthermore DNA based assays are robust, speedy information may be obtained from the little amounts of plant material at any stage of development and it is not affected by environmental conditions. A large number of different molecular techniques are at present available and each of them differs in its informational content. The term 'marker' implies that the loci detected are anonymous and phenotypically neutral. These DNA based markers differentiate the organisms at DNA level and are inherited in simple Mendelian fashion (Waltson, 1993).

They are particularly important for genetic resource management and for the rational use of genetic resources in selection programs. An ideal genetic marker should be polymorphic, multi-allelic, co-dominant, non-epistatic and it should be insensitive to environment (Winter and Kahl, 1995).

Molecular markers are of great importance by being the main source for many genetic analyses and for crop improvement programs. DNA or molecular markers are employed in two broad areas in plant breeding (i) molecular profiling or genome analysis to facilitate the identification of genotypes or assessment of genetic distance and diversity, and (ii) Marker assisted selection (MAS) in plant breeding. Different classes of markers are employed to attain the above-mentioned goals and these include RFLP, AFLP, RAPD, SRAP, SCAR, SSR (Microsatellite).

2.8.2.1a Simple sequence repeats (SSR) markers

Verma *et al.* (2019) conducted an experiment which was carried out using 26 accessions of eggplant including wild relatives aiming to identify stable genotypes of eggplant with tolerant to bacterial wilt. The accessions were characterized using 40 simple sequence repeat (SSR) markers. Thirty-four markers were found polymorphic and produced 136 alleles with an average of four alleles per marker. All the markers have shown amplification across accessions of eggplant-related species. Markers EES-050, EES-065, and EES-066 were highly informative. Markers, EES-031 and EES-051, were specific to the accessions with tolerance to bacterial wilt. Based on the three-year evaluations, the disease incidence (DI) ranged from 1.27% (*S. torvum*) to 72.85% (*S. melongena* var. Kasi Taru). The local eggplant accessions (Bhola Nath, RCMB-10, Singh Nath and RCMBL-1) were found highly stable for yield with lowest wilt incidences when compared to other Indian accessions. The results suggested the incorporation of local accessions/landraces into the eggplant breeding programmes aiming to develop stable cultivars with improved yield and resistant to bacterial wilt.

Zohura and Hoque (2019) conducted an experiment in which attempt was made to search the resistant gene of bacterial wilt in three *Solanum spp.* Some total of 16 different bacterial wilt resistant molecular markers RAPD, SSR, SCAR, SRAP were screened to identify the resistant DNA fragment and only one primer UBC#176 showed amplification in target DNA. The primer UBC#176 gave DNA amplification in target position at 350 bp in the species of *Solanum villosum*. Alternative approach was made to identify resistant gene of bacterial wilt disease. Total RNA was extracted and cDNA was synthesized. The synthesized cDNA was used as template to find out the resistant gene. But none of the gene specific primer was amplified using cDNA as template.

Ghatage and Das (2017) conducted an experiment in which the objective of the investigation were to fingerprint commercial brinjal hybrid and their parental lines using SSR molecular markers to develop an efficient fingerprinting method for seed purity analysis in agriculture production. Brinjal F₁ hybrid 555×RJ and its parents 555 as female and RJ as male were studied for identification and genetic purity testing with high resolution melt analysis and agarose gel electrophoresis. There are 48 brinjal microsatellite loci i.e. SSR primers were used for the analysis. Microsatellite loci, P22 were found to be heteroallelic for parents. P22 identified female specific 180bp repeat and male specific 190bp repeat. However, hybrid (555×RJ) exhibited the alleles of both parents, confirming the heterozygosity of the hybrid with

the presence of two bands at 180bp and 190bp. High resolution melt analysis shows three different melting curves for male, female and hybrid.

Microsatellites are tandem repeats of DNA sequences of only a few base pairs (1-6 base pairs) in length, the most abundant being the dinucleotide repeats. These are most important class of markers which are abundant with long hyper variable region. (CA)_n repeat is one of the most frequently occurring microsatellite in human and many mammalian genomes. In contrast, (AT)_n is more abundant in plants. Variation in the number of tandemly repeated core nucleotide sequences at SSR locus among different genotypes provides the basis for polymorphism that can be used in genetic studies. Their value for genetic analysis lies in their multi-allelism, co-dominant inheritance, relative abundance, genome coverage and suitability for high-throughput PCR-based platforms (Tumbilen *et al.*, 2011).

Demir *et al.* (2010) carried out an experiment in which molecular characterization of eggplant genotypes were collected from different geographical regions of Turkey using SSR and RAPD markers. With amplification of five SSR loci, the number of alleles per microsatellite locus ranged from 2 to 10, with a total of 24 alleles. The greatest number of alleles was found at the *emf21H22* locus (10 alleles); followed by *emh11O01* and *emf21C11* as five and four alleles, respectively. The average number of alleles per locus was 4.8. Using 11 decamer RAPD primers, 100 bands were amplified, among which 29 were polymorphic. The number of bands per primer ranged from seven (*OPH10*, *OPH19*, *OPH20*, *OPH03*) to 14 (*OPB07*). Primer *OPB07* was the most polymorphic, generating 64% polymorphic bands; the rest of the primers gave less than 50% polymorphism. UPGMA dendrograms were used to examine the genetic relatedness of the genotypes.

Sunseri *et al.* (2010) assessed the genetic diversity and evaluated genetic relationships among 70 “scarlet eggplant” (*Solanum aethiopicum* L.) using simple sequence repeat (SSR) analyses. The clusters obtained through cluster analysis did not show any relationships with geographic origins and/or botanical groups. Matrices of genetic similarity from SSR data were utilized in order to obtain a dendrogram.

A large number of SSR based techniques have been developed and a quantum of literature has accumulated regarding the applicability of SSRs in plant genetics and genomics (Kalia *et al.*, 2010).

Nunome *et al.* (2009) constructed simple sequence repeat (SSR) enriched genomic libraries in order to develop SSR markers and sequenced more than

14,000 clones. cDNA libraries were constructed from several eggplant tissues and 6,144 expressed sequence tag (EST) sequences were obtained. From these sequences, 209 primer pairs were designed, 7 of which segregated in the mapping population. On the basis of the segregation data, a linkage map was constructed and the 236 segregating markers were mapped to 14 linkage groups.

Bi-hao *et al.* (2009) reported that, a 762 bp molecular marker linked to a bacterial wilt resistant gene of eggplant was screened by the bulked segregant analysis (BSA) method and sequence characterized amplified region (SCAR) marker linked to bacterial wilt-resistance gene was also obtained.

Wang *et al.* (2008) conducted an experiment in which SSR markers were used to reveal genetic diversity in eggplant. Eighty eight accessions including eighty six of *Solanum melongena* and two wild related species (*Solanum integrifolium* and *Solanum torvum*) were used to analyze the genetic variation with 23 SSR primers.

SSRs tend to cluster at the centromeres and telomeres. However, this problem can be solved by developing SSRs from EST libraries, which are gene rich and more evenly distributed. It was long assumed that SSRs were primarily associated with non-coding DNA, but it has now become clear that they are also abundant in the single and low-copy fraction of the genome. These latter SSRs are commonly referred to as "genic SSRs" or "EST-SSRs" and are present in 1 to 5% of the expressed gene. Genomic SSRs are reported to be collected around particular regions of the chromosomes, such as centromeric areas and the plant DNA sequences deposited in public databases. *S. melongena* having genotype of Angio5 long green is mostly used for development of breeding lines (Stagel *et al.*, 2008).

Morgante *et al.* (2002) reported how co-dominance of alleles, high genomic abundance in eukaryotes and random distribution throughout the genome, with preferential association of low copy region and low quantities of template DNA are required characteristics of SSR markers.

High development cost is a major impediment to the routine application of SSRs in the genetic studies of non-commercial species and for identifying markers located in chromosomal regions of interest. The development of locus-specific SSR markers requires the isolation and characterization of individual loci, a process involving the construction and screening of a DNA library with microsatellite-specific probes, followed by DNA sequencing of positive clones and subsequent PCR primer synthesis and testing. The recovery rate of useful SSRs is generally low due to non-specific amplification and monomorphic loci. In addition, the random selection of clones

prevents predetermination of the chromosomal location and copy number of microsatellite loci. As the flanking DNA is more likely to be conserved, the microsatellite-derived primers can often be used with many varieties and even other species. These markers are easily automated, highly polymorphic and have good analytical resolution, thus making them a preferred choice of markers (Gupta *et al.*, 1994).

2.8.2.1b EST-SSR and other markers

Zohura and Hoque (2019) conducted an experiment in which attempt was made to search the resistant gene of bacterial wilt in three *Solanum spp.* Some total of 16 different bacterial wilt resistant molecular markers RAPD, SSR, SCAR, SRAP were screened to identify the resistant DNA fragment and only one primer UBC#176 showed amplification in target DNA. The primer UBC#176 gave DNA amplification in target position at 350 bp in the species of *Solanum villosum*. Alternative approach was made to identify resistant gene of bacterial wilt disease. Total RNA was extracted and cDNA was synthesized. The synthesized cDNA was used as template to find out the resistant gene. But none of the gene specific primer was amplified using cDNA as template.

Rao *et al.* (2012) conducted an experiment in which thirteen seed samples of eggplants were collected from different parts of the country. A total of 116 polymorphic amplified products were obtained from seven decamer primers, which discriminated all the accessions. The similarity results indicate presence of high levels of genetic diversity in eggplants and a dendrogram constructed by TFPGA methods shows 86.5% polymorphism. Genetically distinct genotypes identified using RAPD markers could be a potential source of germplasm for eggplant's improvement.

Stagel *et al.* (2008) used a set of eggplant EST-SSR markers for phylogenetic analysis and genetic mapping. Since EST-SSRs lie within expressed sequence, they have the potential to serve as perfect markers for genes determining variation in phenotype.

ESTs are relatively conserved region of the genome. ESTs represent informative source of expressed genes and provide a sequence resource that can be exploited for large-scale gene discovery (Boguski *et al.*, 1993). Locus-specific primers flanking EST or genic SSRs can be designed to amplify the microsatellite loci present in the genes. Thus, the generation of (genic) SSR markers is relatively easy and inexpensive because they are a by-product of the sequence data from genes or ESTs that are publicly available.

Chapter-3

Materials and Methods

The present investigation entitled **“Screening of Brinjal Genotypes of North East India against Bacterial Wilt Using SSR Markers”** was carried out in the polyhouse of College of Horticulture and Forestry, Central Agricultural University, Pasighat, Arunachal Pradesh in the year 2019-20. The details of materials and methods used in the investigation are described below:

3.1 Diversity analysis

3.2 Screening of brinjal genotypes against bacterial wilt in field condition

3.3 Assessment of bacterial wilt resistant brinjal genotypes based on molecular analysis.

3.1 Diversity analysis

3.1.1 Experimental site

The polyhouse is situated at the foot hills of Eastern Himalayan Range at an altitude of 153 m above mean sea level, 28°04`N latitude and 95°22`E longitude. The climate of this area is humid, sub-tropical and maximum rainfall occurs between April-September. The soil is sandy loam with pH 6.4 and organic carbon 2.1%. The experiment was conducted in the polyhouse of College of Horticulture and Forestry, Central Agricultural University, Pasighat, Arunachal Pradesh (Figure 3.1.1).

3.1.2 Experimental details

Crop	Brinjal
Design of experiment	Randomize Block Design
Replications	3
Number of genotypes	22
Date of seed sowing	8 th May, 2020
Date of transplanting	12 th June, 2020

3.1.3 Experimental materials

The experimental material for the present study comprised of 20 local genotypes of brinjal including 1 *Solanum torvum* species and 2 check varieties. The list of genotypes along with their sources is given in Table 3.1.

Table 3.1.1 List of brinjal genotypes with their sources

Sl. No.	Genotype	Sources
1.	CHFB-18	Khawzawl (Mizoram)
2.	CHFB-19	Khawzawl (Mizoram)
3.	CHFB-20	Tampi Village (Manipur)
4.	CHFB-21	Tampi Village (Manipur)
5.	CHFB-22	Kodompokpi (Manipur)
6.	CHFB-23	Mebo (Arunachal Pradesh)
7.	CHFB-24	Mebo (Arunachal Pradesh)
8.	CHFB-25	Taki Lalung (Arunachal Pradesh)
9.	CHFB-26	Napit Village (Arunachal Pradesh)
10.	CHFB-27	Napit Village (Arunachal Pradesh)
11.	CHFB-28	Itanagar (Arunachal Pradesh)
12.	CHFB-29	Pasighat (Arunachal Pradesh)
13.	CHFB-30	Boleng (Arunachal Pradesh)
14.	CHFB-31	Maryiang(Arunachal Pradesh)
15.	CHFB-32	Pasighat (Arunachal Pradesh)
16.	CHFB-33	Pasighat(Arunachal Pradesh)
17.	CHFB-34	Village Tuiwandal (Tripura)
18.	CHFB-35	Itanagar (Arunachal Pradesh)
19.	CHFB-36	Pasighat (Arunachal Pradesh)
20.	CHFB-37	Mirem (Arunachal Pradesh)
21.	Arka Nidhi	IIHR(Karnataka)
22.	Arka Keshav	IIHR(Karnataka)

3.1.4 Land preparation

The experiment was conducted under polyhouse of College of Horticulture and Forestry, Central Agricultural University, Pasighat. The nursery was raised well in advance in portrays following with necessary management practices. Five weeks old seedlings were transplanted in pots and light irrigation was given.

Table 3.1.2 Allotment of treatments (genotypes) in pots under RBD

Replication-1	Replication-2	Replication-3
CHFB-18	Arka Nidhi	CHFB-18
CHFB-19	Arka Keshav	CHFB-20
CHFB-20	CHFB-37	CHFB-24
CHFB-21	CHFB-36	CHFB-21
CHFB-22	CHFB-35	CHFB-34
CHFB-23	CHFB-34	CHFB-27
CHFB-24	CHFB-33	CHFB-23
CHFB-25	CHFB-32	CHFB-25
CHFB-26	CHFB-31	CHFB-2
CHFB-27	CHFB-30	CHFB-19
CHFB-28	CHFB-29	CHFB-28
CHFB-29	CHFB-28	CHFB-20
CHFB-30	CHFB-27	CHFB-29
CHFB-31	CHFB-26	CHFB-22
CHFB-32	CHFB-25	CHFB-26
CHFB-33	CHFB-24	CHFB-33
CHFB-34	CHFB-23	CHFB-35
CHFB-35	CHFB-22	CHFB-32
CHFB-36	CHFB-21	Arka Keshav
CHFB-37	CHFB-20	Arka Nidhi
Arka Nidhi	CHFB-19	CHFB-30
Arka Keshav	CHFB-18	CHFB-34

3.1.5 Seed sowing

Seeds were sown in black plastic protrays under polyhouse condition. After the seed sowing, the seeds were covered with a thin layer of the black polythene sheet for maintaining moisture and high humidity till the seed germinated. After which those portrays were watered with rose can whenever required.

3.1.6 Crop management

Fertilizer was applied @ 100 kg N, 60 kg P₂O₅ and 60 kg K₂O per hectare. The required amount of fertilizers were calculated on the basis of plot size and applied through urea (46% N), single superphosphate (16% P₂O₅) and muriate of potash (60% K₂O). One-third dose of nitrogen and entire dose of phosphorus and potash were applied as basal dose and remaining two-third dose of nitrogen was applied in two equal split doses, i.e. 30 and 45 days after transplanting as top dressing. The standard cultural operations like, weeding, irrigation and plant protection measures were adopted whenever needed.

3.1.7 Observations recorded

3.1.7.1 Morphological characters

The observation of following morphological traits will be done as per the IBPGR descriptor.

3.1.7.1a Plant growth habit

It was observed when 50% of the plants bear ripe fruits. The plants of each genotypes were observed prostate, intermediate or upright.

3.1.7.1b Leaf blade lobing

As per the descriptor the leaf blade lobing is generally found very weak, weak, intermediate, strong, very strong.

3.1.7.1c Corolla colour

As per the descriptor the corolla colour is generally found greenish white, white, pale violet, light violet, bluish violet.

3.1.7.1d Fruit colour

As per the descriptor the fruit colour is generally found green, milky white, deep yellow, fire red, scarlet red, lilac grey, purple, purple black and black.

3.1.7.1e. Fruit shape

Various types of fruit shape were observed in the genotypes. The fruit shape observed were round, oval, ovoid, oblong and cylindrical.

3.1.7.1f Spines

The genotypes were recorded on the basis of presence or absence of spines on the stem.

3.1.7.1g Leaf prickles

The number of leaf prickles on upper surface of the leaf observed are none (0), very few (1-2), few (3-5), intermediate (6-10), many (11-20), very many (>20).

3.1.7.1h Pedicel prickles

The number of prickles on surface of pedicel observed as none (0), very few (<3), few (~5), intermediate (~10), many (~20), very many (>30).

3.1.7.1i Fruit Calyx prickles

The number of prickles on surface of fruit calyx observed as none (0), very few (<3), few (~5), intermediate (~10), many (~20), very many (>30).

3.1.7.2 Quantitative characters

The observations were recorded on five randomly selected plants of each genotype and the average was worked out in each replication for different traits. The observations were observed on the following traits as mentioned below:

3.1.7.2a Days to first flowering

Days to first flowering was observed from appearance of flower in the plant population from the date of transplanting.

3.1.7.2b Number of flowers per inflorescence

The total number of flowers present in single cluster of the plant was observed.

3.1.2c Leaf length (cm)

The leaf length was measured with the help of scale to find out the leaf size to denote short, intermediate or wide.

3.1.7.2d Leaf width (cm)

The leaf width was also measured with the help of scale and recorded as narrow, intermediate or wide.

3.1.7.2e Plant height (cm)

Plant height was recorded when the fruit were fully matured and ready for harvesting. It was measured from the base to the highest tip of the plant.

3.1.7.2f Number of primary branches

Number of first order branches arising on main shoot was referred to as primary branches. These were counted at the time of last picking of fruits.

3.1.7.2g Fruit length (cm)

Average fruit length of five mature fruits from each replication was measured from stalk to the bottom end by using a scale and expressed in cm.

3.1.7.2h Fruit girth (cm)

Average fruit girth of five mature fruits were measured by using a digital vernier caliper at the middle of the fruit and expressed in cm.

3.1.7.2i Days to first harvesting

The number of days to first harvest was counted from date of transplanting of seedling to the date of first harvesting.

3.1.7.2j Number of fruits per plant

Total number of fruits harvested from all pickings of individual plant was counted and summed up to get total number of fruits per plant.

3.1.7.2k Fruit weight (g)

The total number of fruits harvested from all pickings of individual plant weighed and the average fruit weight worked out by dividing total weight of fruits from all the pickings with total number of fruits from all pickings and expressed in gram (g).

3.1.7.2l Fruit yield per plant (kg)

The fruit from each picking was recorded from five labeled plants from each replication of every genotype separately.

3.1.8 Statistical analysis

Statistical analysis of the data was carried out by using Statistical Package for Agricultural Research (SPAR 2.0) and Indostat.

3.1.8.1 Mean

On the basis of individual plant observation, the mean for each character in all population was calculated as follows:

$$\bar{Y} = \frac{1}{n} (\sum_{i=1}^n Y_i)$$

Where,

$\bar{Y}$ = Population mean

Y_i = Individual value

n = Number of observations

3.1.8.2 Range

The minimum and maximum value on the basis of individual plant observation was used to indicate the range for a given character.

3.1.8.3 Variance and standard deviation

In all the populations, variance and standard deviation was calculated for all the characters as follows:

$$\text{Variance} = \frac{1}{n-1} \sum (Y_i - \bar{Y})^2$$

Where,

Y_i = Individual value

$\bar{Y}$ = Population value

n = Number of observations

Standard deviation (SD) = $\sqrt{\text{variance}}$

3.1.8.4 Coefficient of variation (CV)

The ratio of standard deviation of a sample to its mean expressed in percentage is called coefficient of variation.

Coefficient of variation (CV) = $\frac{SD}{\bar{X}} \times 100$

Where,

Standard deviation (SD) = $\sqrt{\text{variance}}$

3.1.8.5 Analysis of variance

Analysis of variance (ANOVA) and components of variance for individual character was carried out as per procedure adopted by Singh and Chaudhary (1985).

Structure of ANOVA Table

Source of variation	Degree of freedom	Sum of squares	Mean squares	Expected value of MS	Calculated F
Replications(r)	(r-1)	SSr	$MSr = \frac{RSS}{r-1}$		
Genotypes (g)	(g-1)	SSg	$MSg = \frac{GSS}{g-1}$	$\sigma_e^2 + r \sigma_g^2$	MSg/MSe
Errors (e)	(r-1)(g-1)	SSe	$MSe = \frac{ESS}{(r-1)(g-1)}$	σ_e^2	
Total	(rg - 1)				

Where,

r = Number of replications

g = Number of treatments

Sr = Sum of squares due to replications

Sg = Sum of squares due to treatments

S_e = Sum of squares due to errors

MS_r = Mean sum of squares due to replications

MS_g = Mean sum of squares due to genotypes

MSe = Mean sum of squares due to errors

σ_g^2 = Genotypic variance

σ_e^2 = Error variance

The standard error of mean ($SE_{m\pm}$), Standard error ($SE_{d\pm}$) for genotypes and critical difference (CD) for comparing the means of genotypes were computed as follows:

$$SE_{m\pm} = \sqrt{MSe/r}$$

$$SE_{d\pm} = \sqrt{2MSe/r}$$

CD at 5% = $SE_{(d)} \times t$ value at error degree of freedom at 5% level of significance.

The calculated F values were compared with the tabulated F values at $P=0.05$ and $P=0.01$. If the calculated F value was found higher than the tabulated, it was considered to be significant. All the characters which showed significant differences among the genotypes were subjected further to the analysis of the following parameters:

1. Coefficient of variability (Phenotypic and genotypic)
2. Heritability (%)
3. Genetic advance(GA)
4. Genetic gain (GG)
5. Correlation studies
6. Path coefficient analysis

3.1.8.6. Coefficients of variability (phenotypic and genotypic)

The phenotypic and genotypic coefficient of variability was calculated as per formula given by Burton and de Vane (1953).

Phenotypic coefficient of variability (PCV)

$$PCV (\%) = \frac{\sqrt{\text{Phenotypic variance } (\sigma_p^2)}}{\text{General mean of the character } (\bar{Y})} \times 100$$

Genotypic coefficient of variability (GCV)

$$\text{GCV (\%)} = \frac{\sqrt{\text{Genotypic variance } (\sigma_g^2)}}{\text{General mean of the character } (\bar{Y})} \times 100$$

PCV and GCV were classified as suggested by Sivasubramanian and Menon (1973) as follows:

0-10% = low

10-20% = Moderate

> 20% = High

3.1.8.7 Heritability (h^2)

Heritability (h^2 bs) was calculated as per formula given by Burton and de Vane (1953).

$$\text{Heritability } (h^2 \text{ bs}) \% = \frac{\text{Genotypic variance } (\sigma_g^2)}{\text{Phenotypic variance } (\sigma_p^2)} \times 100$$

Where,

h^2 = Heritability (%)

σ_g^2 = Genotypic variance [$\sigma_g^2 = (Mg - Me) / r$]

σ_p^2 = Phenotypic variance ($\sigma_g^2 + \sigma_e^2$)

Heritability percentage was categorised as demonstrated by Robinson *et al.* (1949).

0 – 30% = Low

30– 60% = Moderate

>60% = High

3.1.8.8 Genetic advance (GA)

The expected genetic advance resulted from selection of 5 percent superior individuals were worked out as suggested by Johnson *et al.* (1955).

$$\text{Genetic advance (GA)} = h^2 (\text{bs}) \times \sigma_p \times k$$

Where,

h^2 = Heritability

σ_p = Phenotypic standard deviation of the character

k = Selection differential at 5 per cent selection intensity i.e. 2.06

3.1.8.9 Genetic gain

Genetic gain is the percentage ratio of genetic advance and population mean as suggested by Johnson *et al.* (1955).

$$\text{Genetic gain (\%)} = \frac{\text{Genetic advance (GA)}}{\text{General mean of the character}} \times 100$$

The genetic advance as percentage of mean was categorised into low, moderate and high as suggested by Johnson *et al.* (1955).

< 20 = Low

20 – 30 = Moderate

>30 = High

3.1.8.10 Analysis of covariance

The analysis of covariance and components of covariance (genotypic and phenotypic) were analyzed between character as per procedure adopted by Singh and Chaudhary (1985).

Source of variation	Degree of freedom	Sum products of between and Y X	Mean sum of products between X and Y	Expected value of mean sum of products between X and Y	Calculated F. value
Replications (r)	r-1	RSP _{xy}	RSP _{xy} /r-1=MSP _r		
Genotypes (g)	g-1	GSP _{xy}	GSP _{xy} /g-1=MSP _g	$\sigma_{e1}^2 + r\sigma_{g1}^2$	MSP _g /MSP _e
Error (e)	(r-1)(g-1)	ESP _{xy}	ESP _{xy} /(r-1)(g-1)=ESP _e	σ_{e1}^2	
Total	(rg-1)				

Where,

r = Number of replications

g = Number of genotypes

MSP_r = Mean sum of products due to replications

MSP_g = Mean sum of products due to genotypes

MSP_e = Mean sum of products due to error

$\sigma_{g1\ g2}$ = Genotypic covariance between character X and Y

$\sigma_{e1\ e2}$ = Error covariance between character X and Y

3.1.8.11 Coefficients of covariance

Genotypic and phenotypic covariances were computed based on expected sum of products from ANOVA table as follows:

$$MSP_g = \sigma_{e1\ e2} + r\sigma_{g1\ g2}$$

$$MSP_e = \sigma_{e1\ e2}$$

$$\text{Hence, } MSP_g = MSP_e + r\sigma_{g1\ g2}$$

$$\text{Genotypic covariance } (\sigma_{g1\ g2}) = \frac{MSP_g - MSP_e}{r}$$

$$\text{Phenotypic covariance } (\sigma_{p1\ p2}) = \sigma_{g1\ g2} + \sigma_{e1\ e2}$$

3.1.8.12 Correlation coefficient

Genotypic (v_g) and phenotypic (v_p) correlation coefficients were estimated according to the formulae given by Al-Jibouri *et al.* (1958).

$$\text{Genotypic correlation } r_{xy}(g) = \frac{Cov_{xy}(g)}{\sqrt{V_x(g) \times V_y(g)}}$$

$$\text{Phenotypic correlation } r_{xy}(p) = \frac{Cov_{xy}(p)}{\sqrt{V_x(p) \times V_y(p)}}$$

Where,

$Cov_{xy}(p)$ = Phenotypic covariance between X and Y character

$Cov_{xy}(g)$ = Genotypic covariance between X and Y character

$V_x(g)$ = Genotypic variance of character X

$V_y(g)$ = Genotypic variance of character Y

$V_x(p)$ = Phenotypic variance of character X

$V_y(p)$ = Phenotypic variance of character Y

The significance of phenotypic and genotypic correlation coefficient was compared with table r values, as given by Fisher and Yates (1963) at n-2 degree of freedom where 'n' denotes number of genotypes.

3.1.8.13 Path coefficients analysis

The path coefficient analysis was done to calculate direct and indirect contribution of different characters towards yield. The direct and indirect effects were calculated by solving the following set of simultaneous equations proposed by Dewey and Lu (1959).

$$\begin{array}{cccccc}
 Py_1 & + Py_2 r_{12} & + Py_3 r_{13} & + \dots\dots\dots & + Py_n r_{1n} & = ry_1 \\
 Py_1 r_{12} & + Py_2 & + Py_3 r_{23} & + \dots\dots\dots & + Py_n r_{2n} & = ry_2 \\
 & - & - & - & - & - \\
 & - & - & - & - & - \\
 Py_1 r_{1n} & + Py_2 r_{2n} & + Py_3 r_{3n} & + \dots\dots\dots & + Py_n & = ry_n
 \end{array}$$

Where,

$Py_1, Py_2, Py_3 \dots\dots\dots, Py_n$ are the direct path effects of 1, 2, 3, ...n variables on dependent variable 'y'

$r_{12}, r_{13}, r_{19}, \dots\dots\dots, r_{(n-1)n}$ are the genotypic coefficients of correlation between various independent variables

$ry_1, ry_2, \dots\dots\dots, ry_n$ are the genotypic correlation coefficients of independents variables with dependent variables 'y'

$Py_1 r_{12}, Py_3 r_{23}, \dots\dots\dots, Py_n r_{2n}$ are the indirect effects.

The variation in the independent variable which remained undetermined by including all variables was assumed to be due to variable (s) not included in the present

investigation. The degree of determination (P^2_{yx}) of such variable (s) on dependent variable was calculated as follows:

$$P^2_{yx} = 1 - (P^2_{y_1} + 2P_{y_1 y_2} r_{12} + 2P_{y_1 y_3} r_{13} + \dots + P^2_{y_2} + 2P_{y_2 y_3} r_{23} + P^2_{y_3} + 2P_{y_3 y_4} r_{34} + \dots + P^2_{y_n})$$

And

$$\text{Residual effect } (R^2) = \sqrt{P^2_{yx}}.$$

3.1.8.14 Genetic divergence

3.1.8.14a Mahalanobis D^2 analysis

Mahalanobis (1936) D^2 statistical analysis was used for assessing the genetic divergence among the test entries. The generalized distance between any two populations is given by formula.

$$D^2 = \sum \sum \lambda_{ij} S_i S_j$$

Where,

D^2 = Square of generalized distance

λ_{ij} = Reciprocal of the common dispersal matrix

$$S^{ai} = (\mu_i - \mu)^2$$

$$S^{aj} = (\mu_j - \mu)^2$$

μ = General mean

Since the formula for computation requires inversion of higher order determinant, transformation of the original correlated unstandardized character means (Xs) to standardized uncorrelated variable (Ys) was done to simplify the computational procedure. The D^2 values were obtained as the corresponding uncorrelated (Ys) values of any two genotypes as per procedure illustrated by Rao (1952).

3.1.8.14b Clustering of D^2 values

All then $(n(n-1)/2)$ D^2 values were clustered using Tocher's method as described by Rao (1952).

3.1.8.14c Intra cluster distance

The intra cluster distances were calculated by the formula given by Singh and Chaudhary (1977).

$$\text{Square of intra cluster distance} = \frac{\sum Di^2}{n}$$

Where,

$\sum Di^2$ = The sum of distance between all possible combinations

N = Number of all possible combinations.

3.1.8.14d Inter cluster distance

The inter cluster distance were calculated by the formulae described by Singh and Chaudhary (1977).

$$\text{Square of inter cluster distance} = \frac{\sum Di^2}{ni \times nj}$$

Where,

$\sum Di^2$ = The sum of distances between all possible combinations ($ni \times nj$) of the entries included in the cluster study.

ni = Number of entries in cluster i

nj = Number of entries in cluster j

3.2 Screening of brinjal genotypes against bacterial wilt in field condition

3.2.1 Experimental site and selection of varieties

Twenty local brinjal genotypes of north eastern India including 1 different species i.e., *Solanum torvum* and two resistant checks i.e., Arka Nidhi and Arka Keshav (IIHR, Bangalore) were used in this experiment. A spacing of 45×60 cm and 10 plants each in 3 replications (Figure 3.2.5.) were used to evaluate during the year 2019-2020 in bacterial wilt sick plot of the polyhouse of College of Horticulture and Forestry, Central Agricultural University, Pasighat, Arunachal Pradesh.

3.2.2 Details of experiment

Brinjal seeds were sown in pro-trays containing well mixed coco-peat, sand and soil and allowed to germinate. 35 days old seedlings were transplanted to bacterial wilt sick plot.

3.2.3 Bacterial inoculum preparation

3.2.3.1 Collection of *Ralstonia* infected samples

Ralstonia solanacearum infected plants were collected from *Ralstonia* infested sick plot (Figure 3.2.1). The presence of pathogen was tested by placing longitudinal sections containing vascular tissue from diseased plants in a test tube with distilled water. The infested tissue showed fine streaks milky ooze and composed of masses of bacteria which come out from the margin of the cut portion within a few minutes (Figure 3.2.3). This distinguishes *Ralstonia* wilt from other wilts like fusarium wilt and physiological wilt. The ooze-out was plated on Triphenyl Tetrazolium Chloride (TTC) media.



Figure 3.2.1 Infected root of diseased plant



a) Infected plant



b) Healthy plant

Figure 3.2.2 Comparison between vascular bundles of an infected plant (a) and a healthy plant (b)



Figure 3.2.3 Bacterial ooze emerging from root of diseased plant into water



Figure 3.2.4 Virulent strain of *Ralstonia solanacearum* and colony formation

3.2.3.2 Preparation of inoculum

Bacterial inoculum was prepared from the virulent (pink colour at centre, irregular shaped and mucoid) colonies (Figure 3.2.4), grown in Triphenyl Tetrazolium Chloride (TTC) media. The composition of Triphenyl Tetrazolium Chloride (TTC) media is shown in (Table 3.2.1.) as follows:

Table 3.2.1 Composition of Triphenyl Tetrazolium Chloride media (1 litre) at pH : 7

Sl. No.	Composition	Quantity
1	Dextrose	10g
2	Peptone	10g
3	Casein	1g
4	Agar	20g
5	Distilled Water	1lit

After autoclaving the media, 5 ml of 1% TTC for 1 litre of media was added maintaining a final concentration of 50mg/ml. Bacterial colonies were seen after 48 hours of incubation at a temperature of 30°C. The bacterial inoculum was harvested and re-suspended in sterile distilled water maintaining an O.D. value of 0.7 at 600nm.

3.2.4 Inoculation of bacteria

35 days old plants were selected for bacterial inoculation. The roots of the seedlings were slightly injured and immersed in the prepared inoculum for about 5 minutes. The seedlings were then transplanted in the sick plot of the polyhouse. The inoculum was also used as soil drench in infecting the soil prior to transplanting containing *Ralstonia solanacearum* population of 50mg/ml.

3.2.5 Observations recorded for screening bacterial wilt

3.2.5.1 Days to 1st incidence of bacterial wilt symptoms after inoculation

3.2.5.2 Plant disease incidence (%)

Number of plants that succumbed due to bacterial wilt was counted at different days after inoculation of bacteria and plant disease incidence was calculated as below:

$$\text{PDI} = \text{No. of infected plants} \times 100 / \text{Total no. of plants assessed.}$$

3.2.5.2a. 1-15 days after inoculation

3.2.5.2b. 16-25 days after inoculation

3.2.5.2c. 26-30 days after inoculation

3.2.5.2d. 30-50 days after inoculation

3.2.5.2e. 50-60 days after inoculation

3.2.5.3 Disease severity index (%)

$$\text{DSI} = \frac{\text{Sum of all disease rating} \times 100}{\text{Total no. of rating} \times \text{Max. disease grade}}$$

Wilt symptoms for each genotype will be graded on a 0-5 scale of Winstead and Kelman (1952). The rating scale is given below:-

0	Highly Resistant	Plants do not show any wilt symptom
1	Resistant	1-20% plants wilted
2	Moderately Resistant	21-40% plants wilted
3	Moderately Susceptible	41-60% plants wilted
4	Susceptible	61-80% plants wilted
5	Highly susceptible	More than 80% plants wilted

3.2.6 Statistical analysis

Statistical analysis of the data was carried out using Statistical Package for Agricultural Research (SPAR 2.0). A dendrogram was made based on the morphological data used in the study.

3.3 Assessment of bacterial wilt resistant brinjal genotypes based on molecular analysis.

3.3.1 Isolation of plant genomic DNA

The genomic DNA was extracted from 22 brinjal genotypes using standardized CTAB method (Murray and Thompson 1980)

3.3.2 Cetyl Trimethyl Ammonium Bromide (CTAB)

In the present study CTAB based extraction buffers used when purifying DNA from plant tissues with some modifications are as follows:

3.3.3 Sample preparation

Recently matured fresh healthy leaves free from any disease infection were selected from all the studied genotypes for the purpose of DNA extraction. The leaves were washed with distill water and wiped with 70 % (v/v) ethanol and then air dried. The leaves were sealed in polythene bags and stored at -80 °C.

Reagents

CTAB buffer (100ml)

- 2.0g CTAB (Hexadecyl trimethyl-ammonium bromide)

- 10ml 1M Tris (pH 8.0): Dissolve 121.1 g of Tris base in 800 ml of H₂O. Adjust pH to 8.0 by adding 42 ml of concentrated HCL. Allow the solution to cool to room temperature before making the final adjustments to the pH. Adjust the volume to 1 L with H₂O. Sterilize using an autoclave.
- 4.0ml 0.5M EDTA (pH 8.0)
- 28.0ml 5M NaCl.
- 40.0 ml sterile distilled water
- 1g PVP-40

Mix all the reagents and adjust the pH to 5.0 with HCL and make up to 100ml with distilled water.

3.3.4 Procedure for isolation and purification of plant genomic DNA

- Grind 200 mg of plant tissue to a fine paste in approximately 500 µl of CTAB buffer with the help of liquid Nitrogen (-196°C).
- Transfer CTAB/plant extract mixture to a microfuge tube and mixed with 75 µl of 10% SDS and 3 µl of β -mercaptoethanol and incubate it for about 15 min-20mins at 65° C in a recirculating water bath.
- The CTAB/plant extract mixture was then cooled down and mixed with 200µl of potassium acetate.
- To each tube add 250 µl of Chloroform: Iso Amyl Alcohol (24:1) and mix the solution by inversion. After mixing, spin the tubes at 12000 rpm for 10 minutes.
- Transfer the upper aqueous phase only (contains the DNA) to a clean microfuge tube.
- To each tube add 0.6 ml volume of ice cold Isopropanol.
- Invert the tubes slowly several times to precipitate the DNA. Generally the DNA can be seen to precipitate out of solution. Alternatively the tubes can be placed for 1 hr at -20° C or overnight after the addition of Isopropanol to precipitate the DNA.
- Following precipitation, the DNA can be extracted by spinning the tube at 13000 rpm for a minute or 10 minutes to form a pellet. Remove the supernatant and wash the DNA pellet by adding two changes of ice cold 70 % ethanol and allow the DNA pellet to dry (approximately 15 min). Do not allow the DNA to over dry or it will be hard to re-dissolve.

- Resuspend the DNA in sterile DNase free water (approximately 50-400 µl H₂O; the amount of water needed to dissolve the DNA can vary, depending on how much is isolated). RNaseA (10 µg/ml) can be added to the water prior to dissolving the DNA to remove any RNA in the preparation (10 µl RNaseA in 10ml H₂O).
- After resuspension, the DNA is incubated at 65°C for 20 min to destroy any DNases that may be present and store at -20°C.
- The quality of DNA was confirmed by using 0.8 per cent agarose gel electrophoresis. Agarose gel electrophoresis of the DNA will show the integrity of the DNA, while spectrophotometry will give an indication of the concentration and cleanliness.

3.3.5 Quantification of DNA

Add 60 µl of each sample to 2940 µl TE or biological grade water, mix well and read at OD₂₆₀ and OD₂₈₀ to determine purity. After UV quantification, adjust the concentration of each DNA sample to 50 ng/µl or a concentration of choice with TE or Biological grade water and store at 4°C.

$$\text{DNA concentration (}\mu\text{g}/\mu\text{l)} = \frac{\text{OD}_{260} \times 50(\text{dilution factor}) \times 50\mu\text{g/ml}}{1000}$$

$$\text{Dilution factor} = \frac{\text{Volume made}}{\text{Volume of the aliquot}}$$

The ratio OD₂₆₀/OD₂₈₀ should be determined to assess the purity of the sample. If the ratio value is between 1.8-2.0, the absorption is probably due to nucleic acids. A ratio less than 1.8 indicates there may be proteins and/or other UV absorbers in the sample, in this case it is advisable to re-precipitate the DNA, a ratio higher than 2.0 indicates that the samples may be contaminated with chloroform or phenol and should be re-precipitated with ethanol.

3.3.6 Quality assessment of DNA

The quality of extracted DNA can be assessed by agarose gel electrophoresis. Electrophoresis is a method used to separate DNA molecules by size. This is achieved by moving negatively charged nucleic acid molecules through a matrix in an electric field.

3.3.7 Reagents

a) 50x TAE buffer

For 1000 ml

Make a 50x stock and dilute to 1x before use

- 242 g Tris base
- 57.1 ml glacial acetic acid
- 100 ml 0.5M EDTA (pH 8.0)

Dissolve the ingredients in a small quantity of distilled water and bring up to 1000 ml with distilled water.

b) Agarose gel (0.8 %)

Weigh out 0.8 g of Analytical Grade (Ultra Pure DNA Grade) Agarose. Add to 100 ml of autoclaved 1x TAE. Heat the solution in order to dissolve the agarose. Let the solution cool to 60 °C. Add 1-2 µl of 10mg/ml ethidium bromide.

c) Gel loading dye (10 ml)

- 25 mg Bromophenol Blue
- 25 mg Xylene Cyanol
- 2.0 ml Glycerol

Make up the volume to 10 ml with sterilized distilled water.

3.3.8 Procedure of agarose gel electrophoresis

a) Pour the melted agarose gel into the gel casting tray (kept in a flat surface) along with the comb.

b) Remove any bubbles in the gel.

c) Allow the gel for 40 minutes to set ensuring that the gel is undisturbed.

d) When the gel has set, remove the comb and transfer to the gel tank.

e) Pour the appropriate amount of 1x TAE buffer into the gel tank so that it covers the gel (it is advisable to use the same batch of TAE buffer in the tank which was used for making the agarose gel).

- f) Mix 5 volumes of PCR product with 1 volume of 6x gel loading dye.
- g) Load samples into the wells in the gel.
- h) Run the gel at the voltage of 80V.
- i) Photograph the gel under ultraviolet light.

3.3.9 PCR based Simple Sequence Repeat (SSR)

The determination of genotypes was carried out using twenty SSR primers which were obtained from the literature search (Table 3.3.1).

3.3.10 Amplification condition of primers

The PCR cycle was carried by an initial denaturation at 94 °C for 3 minutes followed by 40 cycles of

Denaturation at 94 °C for 30 seconds

Annealing: 48 °C to 56.5 °C for 1 minute and

Extension: 72 °C for 1 minute

The final extension was done at 72 °C for 15 minutes followed by holding the PCR products at 4 °C. The annealing temperature varied from primer to primer.

3.3.11 Analysis of genomic SSR primers

Amplification profiles of 20 primers in brinjal population were scored for detection of polymorphism. The primers are mentioned in (Table 3.3.1).

Table 3.3.1 List of SSR primers used for screening

Sl. No.	SSR Loci	Forward Primer (5'-3')	Reverse Primer(3'-5 ')
1	EEMS46	ACCAAACGTGCATGAAACAA	GGAAATGTTGGTGGAATTGG
2	EEMS48	CAATGCAAACAATTATCATTTTCG	TCGATGTTGTTGTCGTCGTT
3	EEMS49	TGAAATTGATCAATACCTATAAATTTAG	GAAAGCCAGGATAGCATTCG
4	EM126	GCATAGCTTATGAGTCAGGTGGCTTT	GCTCATCAAACCATCACATTCAAG
5	EM135	ATCCTGTTGCTGCTCATTTTCCTC	AGGAGGATCCAAGAGGTTTGTGA
6	SSR 1	GTGACTACGGTTTCACTGGT	GATGACGACGACGATAATAGA
7	SSR 2	ATTGAAAGTTGCTCTGCTTC	GATCGAACCCACATCATC
8	SSR 3	CTCTGCTTCACCTCTGTGTT	CCATGAAAGAGAAGATCGAG
9	SSR 4	CACATGGGAACCTACTTACC	GACGACCATCAAACAAGAAT
10	emf21K08	ATCAATGACACCCAAAACCCATTT	GTTTGAAAACCCAATACAAATCCGA
11	emi04H10	ATCGGAGCAAGAGACATTAGATGC	GTTTCCAACACAGTCCCCAATACAACAA
12	emb01D10	AAGAATCGGTCCTCTTTGCATTGT	TGCTTTTCACCTCTCCGCTATCTC
13	emb01J19	GACAGGGATAGGGGTACGGATAGG	ATCCATGTGATGCCTCGATTTTCT
14	eme25D01	AGTCCCAACCAAAATCGTAGAGGC	GTTTCACTGAAGGATGTGGAGTGTGA
15	emg11I04	ACATTTCAGGTTTCTCTTCCCCTC	GTTTGCCCTCCACTCTTGATCATCTTT
16	emg11A06	AGTGCTAATATGCAAGGGGAATGG	GTTTACGGTGATCTTTCCGTATTCCAAA
17	emb01E03	GCGAGAATTTAAAAGGGGGAAGTG	TTGAACCGTCAAGATCCTTCCATT
18	emf21E10	AAGAGAGTCGAGAGAGGTGGATTG	GTTTCTCCTTTGCAATCTCTTTTCA
19	emf01D24	ATACAGTGCCCAACACGATTCAAG	GTTTCAGATAGATGGAAATTAAGGGGGTG
20	emd01E11	ACAGGTCTACCACGAGGCATCTTCT	GTTTGCCCCCAATCCATTAGTGTTAAG

3.3.12 PCR components standardized for amplification

The reaction mixture or master mixture was prepared to a volume of 18.00 µl per sample containing the following components (Table 3.3.2):

Table 3.3.2 Components of master mixture or reaction mixture for SSR

Sl. No.	Reagents	Volume (µl)
1	Reaction buffer (10X)	2.50 µl
2	dNTPs (1mM)	2.50 µl
3	MgCl ₂ (20mM)	2.00 µl
4	<i>Taq</i> DNA polymerase (3U/µl)	0.33 µl
5	Forward Primer (10 pM)	2.00 µl
6	Reverse Primer (10 pM)	2.00 µl
7	Distilled Water	6.67 µl
	Total reaction mixture volume	18.00 µl

The PCR reaction was carried out at a final volume of 20 µl per sample containing 18.00 µl of reaction mixture and 2 µl of 50 ng/µl template DNA.

3.3.13 Agarose gel electrophoresis of amplified PCR products

The agarose gel electrophoresis of amplified PCR products was carried out using following protocol

- 1) Agarose gel (3.0 per cent) was prepared by dissolving 1.8 g of agarose in 60 ml of 1X TAE buffer by heating in a microwave oven until boiling and it was allowed to cool to 60 °C.
- 2) Ethidium Bromide (2.5 µl /60ml) was added to the agarose gel and poured into the gel-casting tray and allowed to set for 40 minutes. The gel casting tray with required number of wells was wiped with 70 % ethanol before pouring the hot agarose gel.
- 3) 10 µl of PCR product was mixed with 5 µl of gel loading dye and loaded into the wells in agarose gel.
- 4) 5 µl of 100bp DNA ladder was loaded into one of the corner lane or well in agarose gel.
- 5) The gel was allowed to run in 1X TAE buffer at 80 volts for 1-1.5 hours.

6) The gel was then visualized under UV-Trans illuminator.

7) The amplified products were compared with the DNA ladder for subsequent study.

3.3.14 Molecular data analysis

3.3.14.1 Scoring and analysis of amplified SSR products

The amplified products obtained from SSR primers were scored for the presence or absence of the products and a matrix of different SSR phenotypes was prepared by scoring them 'zero' for the absence and 'one' for the presence of the loci/marker. Only clear and strong markers were scored. Diffused and/or very weak fragments were not scored as they reported to possess the great propensity for poor reproducibility. The band size was estimated by using a 50 and 100 bp DNA ladder, which was run along with the amplified products. The pair wise similarity matrix between progenies was estimated according to Nei and Li (1979). The similarity coefficient was calculated using the formula as given below

$$S_{ij} = 2a / (2a + b + c)$$

Where S_{ij} is the similarity between two individuals i and j , a is the number of positive peaks shared by both individuals, b is the number of peaks present in i and absent in j and c is the number of peaks present in j and absent in i . The SAHN clustering program was then used to group the entries based on similarity coefficients using the unweighted pair-group method using arithmetic average (UPGMA) using NTSYS software version 2.10.

3.3.14.2 Scoring and analysis of qualitative characters

The above mentioned analysis method was adopted to score qualitative characters in all the twenty two genotypes. The presence of a character was scored as "one" and the absence as "zero".

3.3.14.3 Percentage of polymorphism

The polymorphism in the genotypes was expressed in percentage as shown below.

Percent polymorphism= Total number of polymorphic bands $\times$ 100/ Total number of bands

Percent polymorphism was calculated for each primer and then for all the primers combined together.

3.3.14.4 Efficiency of primers

The efficiency of thirty (30) SSR primers was studied by calculating the following parameters:

Resolving power (Rp) was calculated according to Prevost and Wilkinson (1999) formula

$$Rp = \sum lbi, \text{ where } lbi = 1 - (2 \times 10.5 - pi)$$

Where, 'pi' is the proportion of accessions containing the i^{th} band and lbi is the informativeness of the i^{th} band.

Marker index (MI) of each primer was determined according to Powell et al. (1996), marker index is the product of two functions: DI (Diversity Index) and EMR (Effective Multiplex Ratio)

$$DI = 1 - \sum Pi^2$$

Where Pi is the frequency of i^{th} band

EMR = the product of the fraction of polymorphic loci and the number of polymorphic loci for an individual assay.

Polymorphic information content (PIC) as suggested by Weising *et al.* (2005) and diversity index as suggested by Weir (1996).



Figure 3.1.1 View of Experimental site for diversity analysis



Figure 3.2.5 View of Experimental site for screening of bacterial wilt resistant brinjal genotypes

Chapter-4

Experimental Results

The present investigation entitled “**Screening of Brinjal Genotypes of North East India against Bacterial Wilt Using SSR Markers**” was carried out to evaluate 22 genotypes of brinjal for bacterial wilt resistance and various horticultural traits. The experimental results are presented under the following sub headings:

4.1 Diversity analysis

4.2 Screening of brinjal genotypes against bacterial wilt in field condition

4.3 Assessment of bacterial wilt resistant brinjal genotypes based on molecular analysis.

4.1 Diversity analysis

4.1.1 Morphological characters

4.1.1.1 Plant growth habit

4.1.1.2 Leaf blade lobing

4.1.1.3 Corolla colour

4.1.1.4 Fruit colour

4.1.1.5 Fruit shape

4.1.1.6 Spines

4.1.1.7 Leaf prickles

4.1.1.8 Pedicle prickles

4.1.1.9 Fruit calyx prickles

4.1.2 Quantitative characters

4.1.2.1 Days to 1st flowering

4.1.2.2 Number of flowers per inflorescence

4.1.2.3 Leaf length (cm)

4.1.2.4 Leaf width (cm)

- 4.1.2.5 Plant height (cm)
- 4.1.2.6 No. of primary branches
- 4.1.2.7 Fruit length (cm)
- 4.1.2.8 Fruit girth (cm)
- 4.1.2.9 Days to first harvesting
- 4.1.2.10 Number of fruits per plant
- 4.1.2.11 Fruit weight (kg)
- 4.1.2.12 Fruit yield per plant (kg)

4.1.1 Morphological characters

The various morphological characters of brinjal plant such as plant growth habit, leaf colour, fruit colour and fruit shape was observed. The details for various morphological characters are given below in Table 4.1.1.

4.1.1.1 Plant growth habit

Plant growth habit of collected different genotypes was observed to be upright, intermediate and prostate.

- Upright: CHFB-25, CHFB-27, CHFB-29, CHFB-31, CHFB-33
- Intermediate: CHFB-18, CHFB-19, CHFB-20, CHFB-21, CHFB-22, CHFB-23, CHFB-24, CHFB-28, CHFB-32, CHFB-34, CHFB-35, CHFB-36, CHFB-37, Arka Nidhi, Arka Keshav
- Prostate: CHFB-26, CHFB-30

4.1.1.2 Leaf blade lobing

Leaf blade lobing of different genotypes collected was observed to be very strong, strong, intermediate and weak.

- Very strong: CHFB-33
- Strong: CHFB-22, CHFB-24, CHFB-26, CHFB-27, CHFB-29, CHFB-34, CHFB-35
- Intermediate: CHFB-18, CHFB-19, CHFB-20, CHFB-21, CHFB-23, CHFB-25, CHFB-31, CHFB-37, Arka Nidhi, Arka Keshav
- Weak: CHFB-21, CHFB-28, CHFB-30, CHFB-32, CHFB-36

4.1.1.3 Corolla colour

The corolla colour of the different genotypes collected was recorded as:

- Light violet: CHFB-18, CHFB-19, CHFB-20, CHFB-31, CHFB-33, Arka Nidhi, Arka Keshav
- Bluish violet: CHFB-21, CHFB-22, CHFB-23, CHFB-24, CHFB-25, CHFB-26, CHFB-28, CHFB-29, CHFB-30, CHFB-32, CHFB-34, CHFB-35, CHFB-36, CHFB-37.
- White: CHFB-27

4.1.1.4 Fruit colour

The colour of the mature fruits of different genotypes was recorded as:

- Green: CHFB-18, CHFB-19, CHFB-20, CHFB-21, CHFB-24, CHFB-29, CHFB-30, CHFB-32, CHFB-33, CHFB-34, CHFB-35, CHFB-37
- Purple: CHFB-22, CHFB-23, CHFB-25, CHFB-27, CHFB-36, Arka Nidhi, Arka Keshav
- Purple black: CHFB-26, CHFB-28
- White: CHFB-31

4.1.1.5 Fruit shape

The fruit shape of collected different genotypes was observed to be as follows:

- Round: CHFB-18, CHFB-19, CHFB-24, CHFB-29, CHFB-33, CHFB-34
- Cylindrical: CHFB-20, CHFB-21, CHFB-22, CHFB-23, CHFB-25, CHFB-26, CHFB-27, CHFB-28, CHFB-30, CHFB-31, Arka Nidhi, Arka Keshav
- Oblong: CHFB-32, CHFB-35, CHFB-36, CHFB-37

4.1.1.6 Spines

The presence or absence of the spines on the stem of the genotypes was recorded as:

- Absent: CHFB-18, CHFB-19, CHFB-20, CHFB-21, CHFB-22, CHFB-23, CHFB-24, CHFB-25, CHFB-26, CHFB-27, CHFB-28, CHFB-30, CHFB-31, CHFB-32, CHFB-33, CHFB-36, CHFB-37, Arka Nidhi, Arka Keshav.
- Present: CHFB-29, CHFB-34, CHFB-35

4.1.1.7 Leaf prickles

The prickles on the upper surface of the leaf were recorded as follows:

- None: CHFB- 18, CHFB-19, CHFB-20, CHFB-21, CHFB-22, CHFB-23, CHFB-24, CHFB-25, CHFB-26, CHFB-27, CHFB-28, CHFB-30, CHFB-31, CHFB-32, CHFB-33, CHFB-35, CHFB-36, CHFB-37, Arka Nidhi, Arka Keshav
- Intermediate: CHFB-35
- Many: CHFB-29, CHFB-34

4.1.1.8 Pedicle prickles

The pedicel prickles of different collected genotypes were recorded as mentioned below:

- Few: CHFB-30, CHFB-35
- Intermediate: CHFB-18, CHFB-19, CHFB-24, CHFB-29, CHFB-32, CHFB-34
- None: CHFB-20, CHFB-21, CHFB-22, CHFB-23, CHFB-25, CHFB-26, CHFB-27, CHFB-28, CHFB-31, CHFB-33, CHFB-36, CHFB-37, Arka Nidhi, Arka Keshav

4.1.1.9 Calyx prickles

The calyx prickles of the different genotypes were recorded as follows:

- Few: CHFB-18, CHFB-19, CHFB-24, CHFB-29, CHFB-32, CHFB-34
- Very few: CHFB-26, CHFB-30, CHFB-35
- None: CHFB-20, CHFB-21, CHFB-22, CHFB-23, CHFB-25, CHFB-27, CHFB-28, CHFB-29, CHFB-31, CHFB-33, CHFB-36, Arka Nidhi, Arka Keshav

4.1.2 Quantitative characters

4.1.2.1 Mean performance of genotypes

The analysis of variance for all the traits indicated highly significant variation among the genotypes which revealed the existence of sufficient variability in the germplasm. The mean values obtained for various traits under present study are presented in Table 4.1.2. The character wise mean performance of different genotypes is presented here as below:

4.1.2.1a Days to 1st flowering

Days were calculated from the date of seed sowing to first flowering and it is the desirable traits and showed significant difference among the genotypes. Shortest days to 1st flowering was observed in CHFB-36 (49.71) which was statistically at par with

CHFB-22 (50.32), Arka Keshav (50.79) CHFB-26 (51.76), CHFB-32 (51.81), CHFB-34 (52.20), CHFB-23 (52.35), CHFB-21 (52.55) and CHFB-27 (53.38). Longest days to 50% flowering were counted in CHFB-37 (63.13) with population mean of 58.05 days.

4.1.2.1b Number of flowers per inflorescence

Number of flowers per inflorescence was observed maximum in CHFB-33 (4.00), CHFB-34 (4.00), CHFB-35 (4.00) and Arka Keshav (4.00). The minimum value was reported in CHFB-18 (2.00), CHFB-19 (2.00), CHFB-20 (2.00), CHFB-21 (2.00), CHFB-22 (2.00), CHFB-23 (2.00), CHFB-28 (2.00) and CHFB-29 (2.00) with a population mean of 2.80 flowers per inflorescence.

4.1.2.1c Leaf length

Leaf length was recorded to be maximum in genotypes CHFB-22 (20.53 cm) followed by CHFB-26 (18.77 cm), CHFB-35 (18.67 cm) and CHFB-30 (18.10 cm). The minimum leaf length was recorded in genotypes CHFB-33 (7.97 cm) followed by CHFB-37 (12.77 cm) and CHFB-36 (12.80 cm).

4.1.2.1d Leaf width

Leaf width was recorded to be maximum in genotypes CHFB-27 (13.67 cm) followed by CHFB-29 (12.78 cm) and CHFB-20 (12.68 cm). The minimum leaf width was recorded in genotypes CHFB-33 (5.69 cm) followed by CHFB-37 (7.06 cm), CHFB-36 (7.41 cm) and CHFB-31 (7.51 cm).

4.1.2.1e Plant height (cm)

The data presented clearly showed, significant difference among the genotypes for this trait. Maximum plant height was recorded in CHFB-33 (197.33 cm) which was superior over other genotypes. Minimum plant height was recorded in CHFB-30 (62.33 cm) with general population mean of 120.05 cm.

4.1.2.1f Number of primary branches per plant

Number of primary branches per plant ranged from 4.67 to 10.33 with mean value of 6.71 and all the genotypes differ significantly for number of primary branches per plant. The highest number of branches was recorded in CHFB-31 (10.33) which was statistically almost similar with CHFB-25 (8.67). The lowest number was recorded in CHFB-18 (4.67).

4.1.2.1g Fruit length (cm)

Generally it is assumed that if length of the fruit is more than number of seeds will be more. All the genotypes differ significantly for fruit length. Range for fruit length varied from 1.20 to 18.00 cm with mean value of 9.85 cm. Comparison of mean values of the genotypes CHFB- 25 (18.00 cm) showed maximum fruit length followed by CHFB-26 (17.00 cm) and Arka Keshav (15.00 cm) while minimum fruit length was found in CHFB-33 (1.20 cm).

4.1.2.1h Fruit girth (cm)

All the genotypes differ significantly for fruit girth. Fruit girth for all the genotypes ranged from 1.22 to 8.85 cm with total mean value of 5.88 cm. The maximum fruit breadth was recorded in CHFB-18 (8.85 cm) which was statistically at par with CHFB-19 (8.86 cm), CHFB-34 (7.31 cm) and CHFB-35 (7.32 cm). The minimum fruit breadth was recorded in CHFB-11 (8.86 cm) with population mean of 5.88 cm.

4.1.2.1i Days to first harvesting

All the genotypes were found to have significant differences for days to first harvesting. Days to first harvesting are ranged from 67.00 to 83.00 days with mean value of 73.15 days. The minimum days to first harvesting was recorded in CHFB-31 (67.00) which was statistically at par with CHFB-32 (68.00), CHFB-29 (68.33), CHFB-30 (68.33) and CHFB-21 (69.00). While maximum days to first harvesting was recorded in CHFB-36 (83.00) followed by CHFB-35 (82.33) and CHFB-37 (81.67).

4.1.2.1j Number of fruits per plant

Data recorded on number of fruits per plant showed significant variation among genotypes. Comparison of mean values of the genotypes presented maximum number of fruits per plant in CHFB-31 (33.33) which was significantly superior over all other genotypes. Minimum number of fruits per plant was recorded in CHFB-18 (4.67) with population mean of 9.6.

4.1.2.1k Fruit weight (g)

Fruit weight is another important yield component and needs due consideration, while making selection for yield. All the genotypes differ significantly for fruit weight. Range for fruit weight varied from 3.12 to 507.42 (g) with mean value of 160.51 (g). The maximum fruit weight was observed in CHFB-18 (507.42 g) which was significantly

superior over all other genotypes. The minimum fruit weight was observed in CHFB-33 (3.12 g).

4.1.2.1l Fruit yield per plant (kg)

This character is the primary objective of any breeding programme. Data presented in Table 4.1.2 revealed significant differences among genotypes for fruit yield per plant. Fruit yield per plant ranges from 0.05 to 2.53 (Kg) with mean yield of 1.19 kg. Comparison of data recorded on green fruit yield per plant showed that genotype CHFB-18 (2.53 kg) recorded highest fruit yield per plant followed by CHFB-19 (1.93 kg) and CHFB-26 (1.84 kg) which were significantly superior over other genotypes while minimum fruit yield per plant was recorded in CHFB-33 (0.05 kg).

Table 4.1.1 Morphological character index of brinjal genotypes

Treatment	Plant growth habit	Leaf blade lobing	Corolla colour	Fruit colour	Fruit Shape	Spines	Leaf prickles on upper surface	Pedicle prickles	Calyx prickles
CHFB-18	Intermediate	Intermediate	Light violet	Green	Round	Absent	None	Intermediate	Few
CHFB-19	Intermediate	Intermediate	Light violet	Green	Round	Absent	None	Intermediate	Few
CHFB-20	Intermediate	Intermediate	Light violet	Green	Cylindrical	Absent	None	None	None
CHFB-21	Intermediate	Weak	Bluish violet	Green	Cylindrical	Absent	None	None	None
CHFB-22	Intermediate	Strong	Bluish violet	Purple	Cylindrical	Absent	None	None	None
CHFB-23	Intermediate	Intermediate	Bluish violet	Purple	Cylindrical	Absent	None	None	None
CHFB-24	Intermediate	Strong	Bluish violet	Green	Round	Absent	None	Intermediate	Few
CHFB-25	Upright	Intermediate	Bluish violet	Purple	Cylindrical	Absent	None	None	None
CHFB-26	Prostate	Strong	Bluish violet	Purple Black	Cylindrical	Absent	None	None	Very Few
CHFB-27	Upright	Strong	White	Purple	Cylindrical	Absent	None	None	None
CHFB-28	Intermediate	Weak	Bluish violet	Purple Black	Cylindrical	Absent	None	None	None
CHFB-29	Upright	Strong	Bluish violet	Green	Round	Present	Many	Intermediate	Few
CHFB-30	Prostate	Weak	Bluish violet	Green	Cylindrical	Absent	None	Few	Very Few
CHFB-31	Upright	Intermediate	Light violet	White	Cylindrical	Absent	None	None	None
CHFB-32	Intermediate	Weak	Bluish violet	Green	Oblong	Absent	None	Intermediate	Few
CHFB-33	Upright	Very strong	Light violet	Green	Round	Absent	None	None	None
CHFB-34	Intermediate	Strong	Bluish violet	Green	Round	Present	Many	Intermediate	Few
CHFB-35	Intermediate	Strong	Bluish violet	Green	Oblong	Present	Intermediate	Few	Very Few
CHFB-36	Intermediate	Weak	Bluish violet	Purple	Oblong	Absent	None	None	None
CHFB-37	Intermediate	Intermediate	Bluish violet	Green	Oblong	Absent	None	None	None
Arka Nidhi	Intermediate	Intermediate	Light violet	Purple	Cylindrical	Absent	None	None	None
Arka Keshav	Intermediate	Intermediate	Light violet	Purple	Cylindrical	Absent	None	None	None

Table 4.1.2 Mean performances of twenty two brinjal genotypes for morphological characters

Genotypes	Days to 1st flowering	No. of flowers/infl orescence	Leaf length (cm)	Leaf width (cm)	Plant height (cm)	No. of primary branches	Fruit length (cm)	Fruit girth (cm)	Days to 1st harvesting	No. of fruits/plant	Fruit weight (g)	Fruit yield/plant (kg)
CHFB-18	60.50	2.00	17.77	11.08	134.67	4.67	8.50	8.85	72.00	4.67	507.42	2.53
CHFB-19	59.42	2.00	17.30	12.25	135.33	5.33	8.00	8.76	75.33	6.00	347.61	1.93
CHFB-20	59.49	2.00	17.37	12.68	106.67	6.67	14.00	2.14	72.33	7.33	129.51	0.85
CHFB-21	52.55	2.00	16.97	9.70	105.00	5.67	7.50	2.19	69.00	9.00	107.25	0.90
CHFB-22	50.32	2.00	20.53	12.65	105.67	7.67	10.00	2.06	69.67	7.00	120.30	0.94
CHFB-23	52.35	2.00	17.80	10.51	123.00	7.00	7.00	7.02	69.33	8.33	85.66	0.69
CHFB-24	59.46	3.00	16.60	11.74	129.00	6.67	7.00	5.68	77.00	8.33	109.96	0.81
CHFB-25	56.19	3.00	16.83	8.02	194.67	8.67	18.00	2.06	72.00	10.00	152.26	1.70
CHFB-26	51.76	3.00	18.77	10.97	83.67	8.00	17.00	2.69	71.00	10.67	173.65	1.84
CHFB-27	53.38	2.67	17.00	13.67	113.33	7.33	12.00	2.21	69.33	8.33	97.05	0.82
CHFB-28	60.91	2.00	15.40	10.91	108.67	5.67	6.50	2.07	74.33	11.00	126.30	1.44
CHFB-29	55.99	2.00	16.67	12.78	144.00	6.33	6.50	6.72	68.33	6.00	218.75	1.46
CHFB-30	55.64	3.00	18.10	11.44	62.33	6.67	6.10	5.66	68.33	9.00	71.97	0.50
CHFB-31	58.14	3.00	13.27	7.51	165.00	10.33	8.00	1.37	67.00	33.33	46.72	1.35
CHFB-32	51.81	3.00	15.37	8.58	116.67	5.67	11.00	4.36	68.00	5.00	210.32	1.48
CHFB-33	58.61	4.00	7.97	5.69	197.33	7.67	1.20	1.22	74.33	18.00	3.12	0.05
CHFB-34	52.20	4.00	16.37	10.79	117.33	6.33	7.00	7.31	70.67	5.00	178.82	0.99
CHFB-35	55.36	4.00	18.67	11.43	115.33	5.67	12.00	7.32	82.33	5.00	272.68	1.54
CHFB-36	49.71	3.00	12.80	7.41	102.00	6.67	10.00	4.39	83.00	7.00	196.13	1.10
CHFB-37	63.13	3.00	12.77	7.06	109.00	6.33	12.50	6.05	81.67	6.00	252.11	1.54
Arka Nidhi	56.23	3.00	14.67	10.90	100.33	6.67	12.00	2.19	78.67	12.00	61.26	0.84
Arka Keshav	50.79	4.00	14.87	10.35	72.00	6.00	15.00	2.02	75.67	9.00	62.42	0.87
Mean	55.63	2.80	16.08	10.37	120.05	6.71	9.85	4.29	73.15	9.36	160.51	1.19
C.V.	4.84	8.78	9.65	11.31	8.10	9.63	2.79	9.41	5.88	10.75	10.74	11.26
S.E.	1.55	0.14	0.90	0.68	5.62	0.37	0.16	0.23	2.49	0.58	9.95	0.08
C.D. 5%	4.44	0.41	2.56	1.93	6.03	1.07	0.45	0.66	7.09	1.66	8.40	0.22



CHFB-18-Plant



CHFB-18-Flower



CHFB-18-Fruit



CHFB-19-Plant



CHFB-19-Flower



CHFB-19-Fruit



CHFB-20-Plant



CHFB-20-Flower



CHFB-20-Fruit



CHFB-21-Plant



CHFB-21-Flower



CHFB-21-Fruit

Figure 4.1.1 Glimpse of brinjal genotypes under study



CHFB-22-Plant



CHFB-22-Flower



CHFB-22-Fruit



CHFB-23-Plant



CHFB-23-Flower



CHFB-23-Fruit



CHFB-24-Plant



CHFB-24-Flower



CHFB-24-Fruit



CHFB-25-Plant



CHFB-25-Flower



CHFB-25-Fruit

Figure 4.1.2 Glimpse of brinjal genotypes under study



CHFB-26-Plant



CHFB-26-Flower



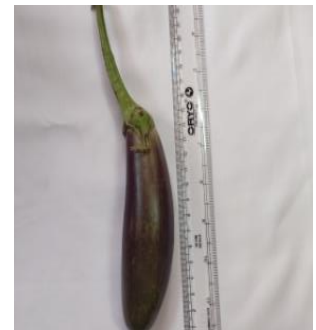
CHFB-26-Fruit



CHFB-27-Plant



CHFB-27-Flower



CHFB-27-Fruit



CHFB-28-Plant



CHFB-28-Flower



CHFB-28-Fruit



CHFB-29-Plant



CHFB-29-Flower



CHFB-29-Fruit

Figure 4.1.3 Glimpse of brinjal genotypes under study



CHFB-30-Plant



CHFB-30-Flower



CHFB-30-Fruit



CHFB-31-Plant



CHFB-31-Flower



CHFB-31-Fruit



CHFB-32-Plant



CHFB-32-Flower



CHFB-32-Fruit



CHFB-33-Plant



CHFB-33-Flower



CHFB-33-Fruit

Figure 4.1.4 Glimpse of brinjal genotypes under study



CHFB-34-Plant



CHFB-34-Flower



CHFB-34-Fruit



CHFB-35-Plant



CHFB-35-Flower



CHFB-35-Fruit



CHFB-36-Plant



CHFB-36-Flower



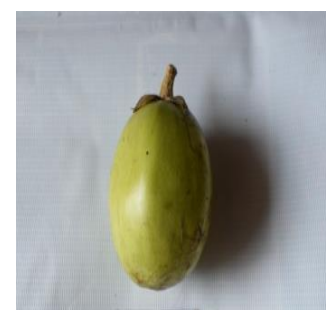
CHFB-36-Fruit



CHFB-37-Plant



CHFB-37-Flower



CHFB-37-Fruit

Figure 4.1.5 Glimpse of brinjal genotypes under study



Arka Nidhi-Plant



G24-Flower



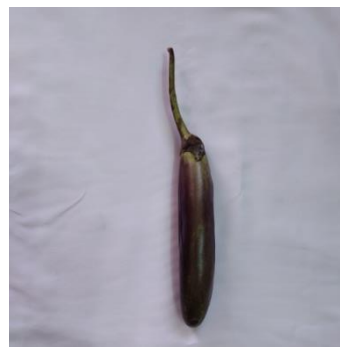
Arka Nidhi-Fruit



Arka Keshav-Plant



Arka Keshav-Flower



Arka Keshav-Fruit

Figure 4.1.6 Glimpse of brinjal genotypes under study

4.1.2.2 Genetic variability components for different morphological characters

In order to determine the scope and response of selection for various traits, the estimates of coefficients of variability at phenotypic (PCV) and coefficient of variability at genotypic (GCV) level, heritability (broad sense), genetic advance presented in the Table 4.1.3, Figure 4.1.7 indicated that PCV was advance as percentage of the mean (genetic gain).

4.1.2.2a Coefficients of variability

The perusal of the data presented in the Table 4.1.3 indicated that PCV of variability were higher in magnitude than their corresponding GCV for all the characters.

Highest phenotypic variance for morphological character was recorded for fruit weight (12952.37) and lowest for fruit yield/plant (0.31) however highest and lowest GCV was recorded for fruit weight (12655.25) and lowest for fruit yield/plant (0.29) respectively.

The highest estimates of phenotypic and genotypic coefficient of variation was observed for fruit weight (PCV=70.90%, GCV=70.08%) followed by No. of fruits/plant (PCV=66.20%, GCV=65.32%) and fruit girth (PCV=60.16%, GCV=59.42%). The lowest estimates of PCV and GCV were recorded in case of days to 1st flowering (PCV=8.06%, GCV= 6.44%) and days to first harvesting (PCV=8.20%, GCV= 13.02%).

4.1.2.2b Heritability (%)

Heritability (bs) estimates ranged from 89.50% to 48.50%. Higher heritability was recorded for fruit length (89.90%) followed by fruit weight (87.70%) and fruit girth (87.60%) however, lowest heritability was recorded for days to 1st harvesting (48.50%) followed by days to 1st flowering (64.00%) and number of flowers/inflorescence (69.50%).

4.1.2.2c Genetic advance

In the present investigation, genetic advance was high for fruit weight (229.06%). However, observation recorded lower in case of fruit yield/plant (1.08%) followed by number of flowers per inflorescence (1.40%) and number of primary branches (2.13%) respectively.

4.1.2.2d Genetics advance as percentage of mean

The genetic advance expressed as percent of population mean is genetic gain in which it was high for fruit weight (142.71%) followed by number of fruit/plant (132.78%), fruit girth (120.90%). However, days to 1st harvest (8.19%) followed by days to 1st flowering (10.62%) and leaf length (27.39%) showed least genetic gain from the present investigation.

The mean performance of twenty two brinjal genotypes were observed and presented in the Table 4.1.3. Significantly early flowering was observed in the genotype CHFB- 36 (49.71 days) followed by genotypes CHFB-22 (50.32 days) and in the check variety Arka Keshav (50.79 days) where, genotype CHFB-37 observed late flowering after 63.13 days after sowing. No. of flowers per inflorescence was observed significantly higher in genotypes CHFB-33, CHFB-34, CHFB-35 and Arka keshav observed four flowers per inflorescence. Leaf length, leaf width was observed significantly higher in CHFB-22 (20.53) and CHFB-27 (13.67) respectively while least leaf length (7.97) and width (5.69) was observed in the genotypes CHFB-33. Genotypes CHFB-33 recorded highest plant height (197.33cm) followed by CHFB-25 (194.67 cm) showed significant among all the genotypes where genotype CHFB-30 observed lowest (62.33 cm). No. of primary branches counted significantly maximum (10.33) in CHFB-31 while minimum was found in CHFB-18 (4.67). The great variation was observed in the ease of fruit length and fruit girth in case of these genotypes collected from North east states of India as well as varieties used as checked collected from IIHR, Bangalore. Minimum fruit length (1.20 cm) and girth (1.22 cm) were observed in CHFB-33 while maximum fruit length (18.00 cm) was observed in CHFB-25 followed by CHFB-26 (17.00cm) which indicates significant among all the genotypes.

Days to first harvest observed at par in all the genotypes. The early harvesting was done 67 days after transplanting in case of genotype CHFB-31 while late harvesting (83.00 days) was done in CHFB-37 followed by CHFB-35 and CHFB-37.

Number of fruits per plant and fruit weight is the major component responsible for direct increasing the yield of crops. Maximum number of fruits per plant (33.33) was noted in the genotype CHFB-31 which was significantly higher among all the genotypes while, minimum numbers recorded in CHFB-18 (4.67). The genotypes collected for investigation observed significant variation and ranged from 3.12-7.42 where maximum fruit weight (507.42 g) in the genotype CHFB-18 while minimum fruit weight (3.12 g), it

may be due to local types showing small in size. Maximum fruit yield per plant was recorded (2.53 kg) in the genotypes CHFB- 18 followed by CHFB-19 (1.93 kg) which showed significant among all genotypes while minimum fruit yield (0.05 kg) was recorded in the genotypes CHFB-33.

Table 4.1.3 Genetic variability components for different morphological characters of twenty two brinjal genotypes

Sl. No.	Characters	Range		Variance		Coefficient of variability (%)		Heritability %	Genetic advance	Genetic advance as % of mean (Genetic gain)
		Min	Max	Phenotypic	Genotypic	PCV	GCV			
1.	Days to 1st flowering	49.71	63.13	20.12	12.87	8.06	6.44	64.00	5.91	10.62
2.	No. of flowers/ inflorescence	2.00	4.00	0.57	0.51	27.08	25.62	69.50	1.40	49.93
3.	Leaf length (cm)	7.97	20.53	8.73	6.32	18.37	15.63	72.40	4.40	27.39
4.	Leaf width (cm)	5.69	13.67	5.47	4.09	22.55	19.51	74.90	3.60	34.77
5.	Plant height (cm)	62.33	197.33	1172.55	1077.94	28.52	27.35	81.90	64.84	54.02
6.	No. of primary branches	4.67	10.33	1.81	1.39	20.07	17.61	77.00	2.13	31.84
7.	Fruit length (cm)	1.20	18.00	15.92	15.85	40.49	40.40	89.50	8.18	83.03
8.	Fruit girth (cm)	1.22	8.85	6.65	6.49	60.16	59.42	87.60	5.18	120.90
9.	Days to 1st harvesting	67.00	83.00	35.98	17.46	8.20	5.71	48.50	5.99	8.19
10	No. of fruits/plant	4.67	33.33	38.43	37.41	66.20	65.32	87.40	12.43	9.36
11.	Fruit weight (g)	3.12	507.42	12952.37	12655.25	70.90	70.08	87.70	229.06	160.51
12.	Fruit yield/plant (kg)	0.05	2.53	0.31	0.29	47.15	45.79	84.30	1.08	1.18

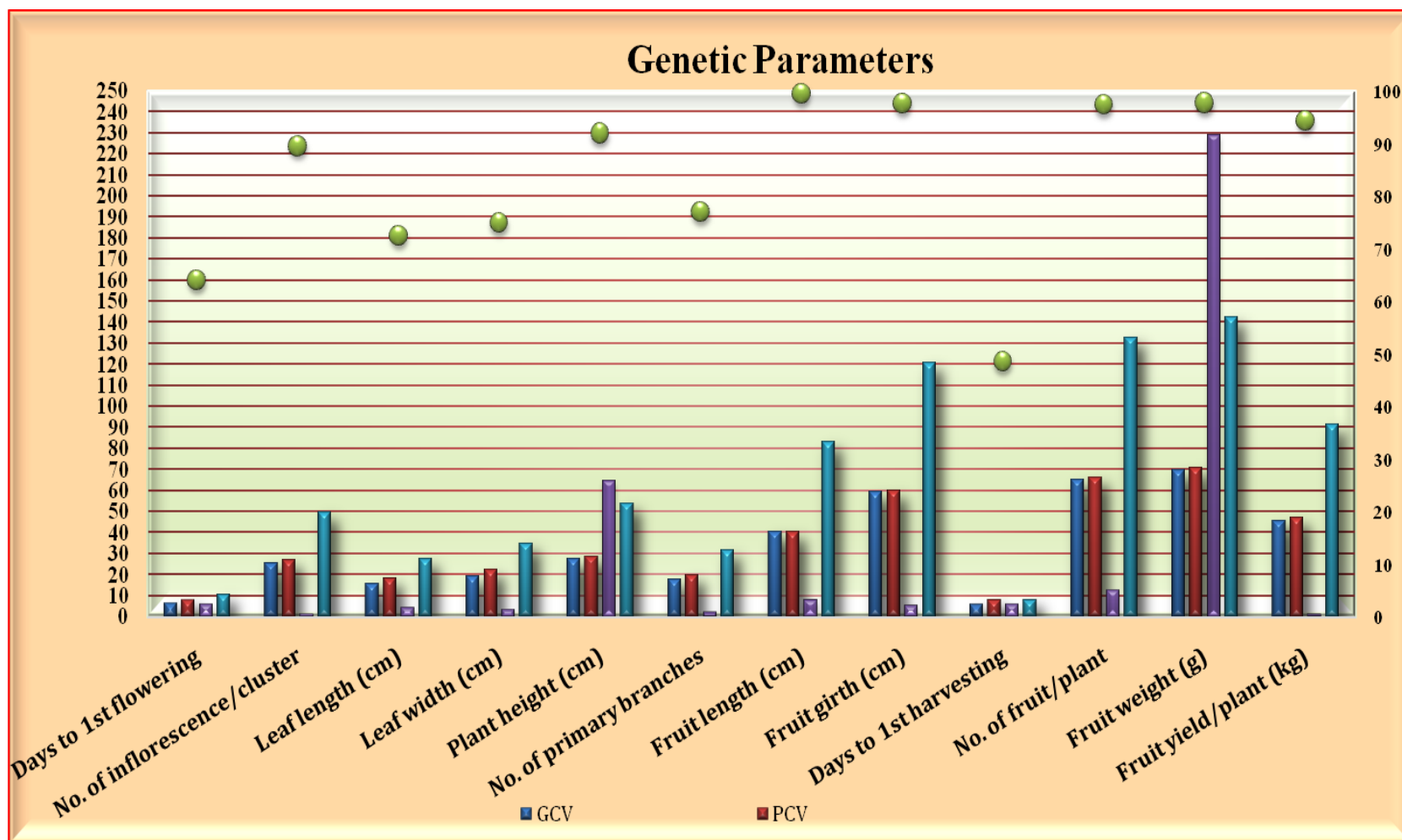


Figure 4.1.7 Genetic variability components for different morphological characters of twenty two brinjal genotypes

4.1.2.3 Correlation studies for different morphological characters

Correlation coefficient of twenty two brinjal genotypes for twelve characters was studied in all possible combinations shown in the Table 4.1.4 and Table 4.1.5. As the data showed in the Table 4.1.5, the values of genotypic correlation coefficient revealed higher in magnitude than the phenotypic correlation coefficient values.

4.1.2.3a Days to 1st flowering

Positive significant correlation coefficient of days to first flowering was observed with plant height (0.293) and days to first harvesting (0.283) and it was positive non significantly correlated with fruit girth (0.172), number of fruits per plant (0.158), fruit weight (0.212) and fruit yield (0.210) while, the characters such as number of flowers per inflorescence (-0.172), leaf length (-0.214), leaf width (-0.104), number of primary branches (-0.049) and fruit length (-0.187) recorded non significant negative correlation with days to first flowering at phenotypic level (r_p).

However, at genotypic level, positive significant correlation coefficient of days to first flowering was observed with plant height (0.404), fruit weight (0.306) and fruit yield per plant (0.283) and it was positive non significantly correlated with fruit girth (0.193), number of fruits per plant (0.178) and days to first harvesting (0.171). While days to first flowering had shown negative significant correlation with leaf length (-0.304) and negative non-significant correlation with leaf width (-0.083) and number of primary branches (-0.149).

4.1.2.3b Number of flowers per inflorescence

Number of flowers per inflorescence had significant positive correlation with days to first harvesting (0.245) and non significant positive correlation with plant height (0.41), number of primary branches (0.143), fruit length (0.093) and number of fruits per plant (0.161). However highly significant negative correlation of number of flowers per inflorescence was recorded with leaf length (-0.374), and leaf width (-0.350) at phenotypic level.

Number of flowers per inflorescence had positive and significant genotypic correlation with days to first harvesting (0.446) and non significant positive correlation with number of primary branches (0.192), fruit length (0.098), number of fruits per plant (0.165) and plant height (0.042). However highly significant negative correlation of number of

flowers per inflorescence was recorded with leaf length (-0.476), leaf width (-0.472), fruit yield per plant (-0.297) and fruit weight (-0.274) whereas, negative non significant correlation with fruit girth (-0.108) at genotypic level (r_g).

4.1.2.3c Leaf length (cm)

Leaf length had highly significant positive phenotypic correlation (r_p) with leaf width (0.728), fruit length (0.302), fruit yield/plant (0.282), fruit weight (0.280) and fruit girth (0.273) whereas, negative and significant correlation with number of fruits/plant (-0.418) and plant height (-0.349) at phenotypic level (r_p).

However, at genotypic correlation (r_g) leaf length had positive and significantly correlated with leaf width (0.814), fruit yield/plant (0.358), fruit length (0.349), fruit weight (0.323) and fruit girth (0.318) while, negative and significant correlation showed for number of fruits per plant (-0.493), plant height (-0.449) and days to 1st harvesting (-0.410) and negative correlation was observed with number of primary branches (-0.144).

4.1.2.3d Leaf width (cm)

Leaf width had positive correlation at phenotypic level (r_p) with fruit length (0.086), fruit girth (0.218), fruit weight (0.155) and fruit yield per plant (0.085). However, leaf width showed highly significant negative correlation with plant height (-0.366) and number of fruits per plant (-0.384) while, number of primary branches (0.267) and days to first harvesting (-0.202) had significant negative correlation and non significant negative correlation with leaf width respectively.

However, leaf width at genotypic level (r_g) showed positive and significant correlation with fruit girth (0.269) and positive non-significant correlation with fruit weight (0.184), fruit length (0.107) and fruit yield (0.091) whereas, number of fruits per plant (-0.473), plant height (-0.469), number of primary branches (-0.345) and days to 1st harvest (-0.263) had negative and significant genotypic correlation (r_g).

4.1.2.3e Plant height (cm)

Plant height had highly significant positive correlation with number of fruits per plant (0.385) and number of primary branches (0.357). The fruit girth (0.030) and fruit yield per plant (0.092) had non-significant positive correlation, while fruit length (-0.236), fruit girth (-0.054) and days to first harvesting (-0.103) had non-significant negative correlation with plant height.

Plant height had highly significant positive correlation with number of primary branches (0.411) and number of fruits per plant (0.401) and non significant positive correlation with fruit yield per plant (0.092), and fruit weight (0.028) while, negative and significantly correlated with fruit length (0.246) and negative non-significantly correlated with fruit girth (-0.060), and days to first harvesting (-0.118).

4.1.2.3f Number of primary branches

In the present investigation, it was found that highly significant and positive phenotypic correlation with number of fruits per plant (0.688). However fruit length (0.148) had positive non significant correlation with the above traits. The characters such as fruit girth (-0.528) and fruit weight (-0.253) and days to first harvesting had non-significant negative correlation.

It was positive and significantly correlated with number of fruits per plant (0.802) and positive non significantly correlated with fruit length (0.164). But negative and significantly correlated with fruit weight (-0.600), fruit girth (-0.592), days to 1st harvesting (-0.388) and fruit yield per plant (-0.245) at genotypic level (r_g).

4.1.2.3g Fruit length (cm)

Fruit length showed significant positive correlation with fruit yield per plant (0.365) whereas days to first harvesting (0.142) and fruit weight (0.108) had non-significant positive correlation and fruit girth (-0.241) and number of fruits per plant (0.187) had non-significant negative correlation.

However, fruit length had shown positive and significant correlation with fruit yield/plant (0.376) and positive non-significant correlation with fruit weight (0.108) and days to first harvesting (0.205). Whereas, it had shown negative and significant correlation with fruit girth (-0.244) and negative non-significant correlation with number of fruits per plant (-0.188) at genotypic level (r_g).

4.1.2.3h Fruit girth (cm)

Fruit girth had highly significant positive correlation with fruit weight (0.725) and fruit yield per plant (0.423) while non significant positive correlation with days to first harvesting (0.14). The fruit girth also had highly negative significant correlation with number of fruits per plant (-0.531).

At genotypic level (r_g), fruit girth had shown positive and significant correlation with fruit weight (0.736) and fruit yield per plant (0.438) and positive non significant correlation with days to first harvesting (0.191). Whereas, negative and significant correlation with number of fruits per plant (-0.543) at genotypic level (r_g).

4.1.2.3i Days to 1st harvesting (cm)

In the present investigation, days to first harvesting showed non significant positive correlation with fruit weight (0.163) and fruit yield per plant (0.049). While number of fruits per plant was recorded non significant negative correlation with the above traits.

At genotypic level (r_g), days to 1st harvesting showed positive significant correlation with fruit weight (0.245) and positive non significant correlation with fruit yield per plant (0.089). While, showed negative and significant correlation with number of fruits per plant (-0.282).

4.1.2.3j Number of fruits per plant

Number of fruits per plant had highly significant negative correlation with fruit weight (-0.515) and non significant negative correlation with fruit yield per plant (-0.197) under the investigation of phenotypic level.

However, at genotypic level (r_g) number of fruits per plant had shown negative and significant correlation with fruit weight (-0.524) and negative non significant correlation with fruit yield per plant (-0.218).

4.1.2.3k Fruit weight (kg)

In the present observation, fruit weight noted highly significant and positive correlation with fruit yield per plant (0.818).

At genotypic level (r_g) fruit weight showed positive and significant correlation with fruit yield per plant (0.856) while negative and significant correlation was not recorded at genotypic level (r_g).

Table 4.1.4 Phenotypic correlation coefficients (r_p) of twenty two morphological traits of brinjal genotypes

Correlation Matrix	Days to 1st flowering	No. of flowers/ inflorescence	Leaf length (cm)	Leaf width (cm)	Plant height (cm)	No. of primary branches	Fruit length (cm)	Fruit girth (cm)	Days to 1st harvesting	No. of fruits/plant	Fruit weight (g)	Fruit yield/plant (kg)
Days to 1st flowering	1.000	-0.172	-0.214	-0.104	0.294 *	-0.049	-0.187	0.173	0.283 *	0.158	0.212	0.210
No. of flowers/inflorescence		1.000	-0.374 **	-0.350 **	0.041	0.1431	0.093	-0.097	0.245 *	0.161	-0.252 *	-0.258*
Leaf length (cm)			1.000	0.728 ***	-0.350 **	-0.144	0.303 *	0.273 *	-0.195	-0.419 ***	0.281 *	0.282*
Leaf width (cm)				1.000	-0.366 **	-0.267 *	0.086	0.218	-0.202	-0.384 **	0.155	0.085
Plant height (cm)					1.000	0.357 **	-0.236	-0.054	-0.103	0.385 **	0.023	0.092
No. of primary branches						1.000	0.148	-0.528 ***	-0.190	0.688 ***	-0.533 ***	-0.253*
Fruit length (cm)							1.000	-0.241	0.142	-0.187	0.108	0.365*
Fruit girth (cm)								1.000	0.140	-0.531 ***	0.725 ***	0.423**
Days to 1st harvesting									1.000	-0.198	0.163	0.0487
No. of fruit/plant										1.000	-0.515 ***	-0.197
Fruit weight (g)											1.000	0.818**
Fruit yield/plant (kg)												1.000

Table 4.1.5 Genotypic correlation coefficients (r_p) of twenty two morphological traits of brinjal genotypes

Correlation Matrix	Days to 1st flowering	No. of flowers/inflorescence	Leaf length (cm)	Leaf width (cm)	Plant height (cm)	No. of primary branches	Fruit length (cm)	Fruit girth (cm)	Days to 1st harvesting	No. of fruits/plant	Fruit weight (g)	Fruit yield/plant (kg)
Days to 1st flowering	1.000	-0.200	-0.304*	-0.083	0.404**	-0.149	-0.233	0.193	0.171	0.178	0.306*	0.283*
No. of flowers/inflorescence		1.000	-0.476**	-0.472**	0.042	0.192	0.098	-0.108	0.446**	0.165	-0.274*	-0.297*
Leaf length (cm)			1.000	0.814**	-0.449**	-0.2199	0.349*	0.318*	-0.410**	-0.493**	0.323*	0.358*
Leaf width (cm)				1.000	-0.469**	-0.345*	0.107	0.269*	-0.263*	-0.473**	0.184	0.091
Plant height (cm)					1.000	0.411**	-0.246*	-0.06	-0.118	0.401**	0.028	0.092
No. of primary branches						1.000	0.164	-0.592**	-0.388*	0.802**	-0.600**	-0.245*
Fruit length (cm)							1.000	-0.244*	0.205	-0.188	0.108	0.376*
Fruit girth (cm)								1.000	0.191	-0.543**	0.736**	0.438**
Days to 1st harvesting									1.000	-0.282*	0.245*	0.089
No. of fruits/plant										1.000	-0.524**	-0.218
Fruit weight (g)											1.000	0.856**
Fruit yield/plant (kg)												1.000

4.1.2.4 Path coefficient analysis

Path coefficient analysis devised by Dewey and Lu (1959). However, it provides a realistic basis for allocation of appropriate weightage to various attributes, while designing a pragmatic programme for the improvement of yield. The recorded data on path coefficient analysis both at phenotypic and genotypic level showing the direct and indirect effects of significant traits on fruit yield/plant. The results had shown in Table 4.1.6, Figure 4.1.8, Table 4.1.7 and Figure 4.1.9 respectively.

4.1.2.4a Path coefficient analysis at phenotypic level

A perusal of phenotypic path coefficient analysis was carried out of 22 genotypes of brinjal for twelve characters where maximum direct positive effect on fruit yield/plant was imposed by fruit weight (0.858) followed by number of fruits/plant (0.443), fruit length (0.401), leaf length (0.080), plant height (0.052), fruit girth (0.038) and days to first harvesting (0.018) while maximum negative direct effects on fruit yield per plant were recorded for number of primary branches (-0.157), number of flowers per inflorescence (-0.094), days to 1st harvesting (-0.066) and leaf width (-0.045) respectively.

The maximum and positive indirect effect on fruit yield/plant was participated by fruit yield per plant through fruit weight (0.818), fruit weight through fruit girth (0.622), fruit yield per plant through fruit length (0.365), number of fruits/plant through number of primary branches (0.305), fruit weight through leaf length (0.241), fruit yield per plant through days to 1st flowering (0.210), fruit weight through days to 1st flowering (0.182), number of fruits/plant through plant height (0.170), fruit weight through days to 1st harvesting (0.140), fruit weight through leaf width (0.133), fruit length through leaf length (0.121), number of primary branches through fruit weight (0.084), number of primary branches through fruit girth (0.083), leaf length through leaf width (0.058), leaf length through days to 1st harvesting (0.057) and plant height through number of fruits per plant (0.020).

The characters like fruit weight had maximum negative indirect effect on fruit yield per plant through number of primary branches (-0.457) and number of fruits per plant (-0.442) and number of flowers per inflorescence (-0.216). The characters like number of fruits per plant imposed negative indirect effect through fruit girth (-0.235), fruit weight (-0.228), leaf length (-0.185), leaf width (-0.169), days to 1st harvesting (-0.088) and fruit length through days to 1st harvesting (-0.075). Residual effect at phenotypic level was also observed to be 0.368 from the present investigation.

4.1.2.4b Path coefficient analysis at genotypic level

Fruit weight (0.925) had maximum positive direct effect on fruit yield per plant followed by number of fruits per plant (0.460), leaf length (0.402), fruit length (0.275), plant height (0.070), days to 1st flowering (0.039), days to 1st harvesting (0.021). However, maximum negative direct effect on fruit yield per plant was imposed by leaf width (-0.228), number of primary branches (-0.157), fruit girth (-0.098) and number of flowers per inflorescence (-0.047).

The characters like fruit yield per plant had maximum positive indirect effect on fruit yield per plant through fruit weight (0.856), fruit girth (0.438), fruit length (0.376), leaf length (0.358). The maximum and positive indirect effect on fruit yield per plant was participated by fruit weight through fruit girth (0.681), leaf length (0.298), days to 1st flowering (0.283), days to 1st harvesting (0.229), leaf length through leaf width (0.328), fruit length (0.140) and fruit weight (0.130), number of fruits per plant through number of primary branches (0.370), leaf width through number of flowers per inflorescence (0.107), leaf width through plant height (0.107) and leaf width through number of fruits per plant (0.107).

The characters like fruit weight imposed high negative indirect effect through number of primary branches (-0.555), number of fruits per plant (-0.484) and number of flowers per inflorescence (-0.253). Number of fruits per plant also imposed negative indirect effect through fruit girth (-0.250), fruit weight (-0.240), leaf length (-0.227) and leaf width (-0.217), leaf length through number of fruits per plant (-0.199), number of flowers per inflorescence (-0.191), plant height (-0.180), leaf width through leaf length (-0.186). Residual effect at genotypic level was also observed to be 0.231 from the present investigation.

Table 4.1.6 Direct (diagonal) and indirect effects of morphological characters on fruit yield per plant at phenotypic level in brinjal genotypes

Path Matrix	Days to 1st flowering	No. of flowers/inflorescence	Leaf length (cm)	Leaf width (cm)	Plant height (cm)	No. of primary branches	Fruit length (cm)	Fruit girth (cm)	Days to 1st harvesting	No. of fruits/plant	Fruit weight (g)	Fruit yield/plant (kg)
Days to 1st flowering	0.018	-0.003	-0.004	-0.002	0.005	-0.001	-0.003	0.003	0.005	0.003	0.004	0.210
No. of flowers/inflorescence	0.016	-0.094	0.035	0.033	-0.004	-0.013	-0.009	0.009	-0.023	-0.015	0.024	-0.258*
Leaf length (cm)	-0.017	-0.030	0.080	0.058	-0.028	-0.012	0.024	0.022	-0.016	-0.034	0.022	0.282*
Leaf width (cm)	0.005	0.016	-0.033	-0.045	0.017	0.012	-0.004	-0.010	0.009	0.017	-0.007	0.085
Plant height (cm)	0.0153	0.002	-0.018	-0.019	0.052	0.019	-0.012	-0.003	-0.005	0.020	0.002	0.092
No. of primary branches	0.008	-0.023	0.023	0.042	-0.056	-0.157	-0.023	0.083	0.030	-0.108	0.084	-0.253*
Fruit length (cm)	-0.075	0.037	0.121	0.035	-0.095	0.059	0.401	-0.097	0.057	-0.075	0.043	0.365*
Fruit girth (cm)	0.007	-0.004	0.010	0.008	-0.002	-0.020	-0.009	0.038	0.005	-0.020	0.028	0.423**
Days to 1st harvesting	-0.019	-0.016	0.0130	0.013	0.007	0.013	-0.009	-0.009	-0.066	0.013	-0.011	0.049
No. of fruits/plant	0.070	0.071	-0.185	-0.170	0.170	0.305	-0.083	-0.235	-0.088	0.443	-0.228	-0.197
Fruit weight (g)	0.182	-0.216	0.241	0.133	0.025	-0.457	0.093	0.622	0.140	-0.442	0.858	0.818**
Fruit yield/plant (kg)	0.210	-0.258	0.282	0.085	0.092	-0.253	0.365	0.423	0.049	-0.197	0.818	1.000
Partial R2	0.004	0.024	0.023	-0.004	0.005	0.040	0.146	0.016	-0.003	-0.087	0.701	

Table 4.1.7 Direct (diagonal) and indirect effects of morphological characters on fruit yield per plant at genotypic level in brinjal genotypes

Path Matrix	Days to 1st flowering	No. of flower s/inflo rescence	Leaf length (cm)	Leaf width (cm)	Plant height (cm)	No. of primary branches	Fruit length (cm)	Fruit girth (cm)	Days to 1st harvesting	No. of fruits/plant	Fruit weight (g)	Fruit yield/plant (kg)
Days to 1st flowering	0.039	-0.008	-0.012	-0.003	0.016	-0.006	-0.009	0.008	0.007	0.007	0.012	0.283*
No. of flowers/inflorescence	0.009	-0.047	0.023	0.022	-0.002	-0.009	-0.005	0.005	-0.021	-0.008	0.013	-0.297*
Leaf length (cm)	-0.122	-0.191	0.402	0.328	-0.180	-0.088	0.140	0.128	-0.165	-0.199	0.130	0.358*
Leaf width (cm)	0.019	0.108	-0.186	-0.228	0.107	0.079	-0.025	-0.061	0.060	0.108	-0.042	0.091
Plant height (cm)	0.028	0.003	-0.031	-0.033	0.070	0.029	-0.017	-0.004	-0.008	0.028	0.002	0.092
No. of primary branches	0.024	-0.030	0.035	0.054	-0.065	-0.157	-0.026	0.093	0.061	-0.126	0.094	-0.245*
Fruit length (cm)	-0.064	0.027	0.096	0.030	-0.068	0.045	0.275	-0.067	0.056	-0.052	0.030	0.376*
Fruit girth (cm)	-0.019	0.011	-0.031	-0.026	0.006	0.058	0.024	-0.098	-0.019	0.053	-0.072	0.438**
Days to 1st harvesting	0.004	0.010	-0.009	-0.006	-0.003	-0.008	0.004	0.004	0.021	-0.006	0.005	0.089
No. of fruit/plant	0.082	0.076	-0.227	-0.217	0.185	0.369	-0.087	-0.250	-0.130	0.460	-0.241	-0.218
Fruit weight (g)	0.283	-0.254	0.298	0.170	0.026	-0.555	0.100	0.681	0.229	-0.484	0.925	0.856**
Fruit yield/plant (kg)	0.283*	-0.297*	0.358*	0.091	0.092	-0.245*	0.376*	0.438**	0.089	-0.218	0.856* *	1.00
Partial R2	0.011	0.014	0.144	-0.021	0.006	0.039	0.103	-0.043	0.002	-0.101	0.792	

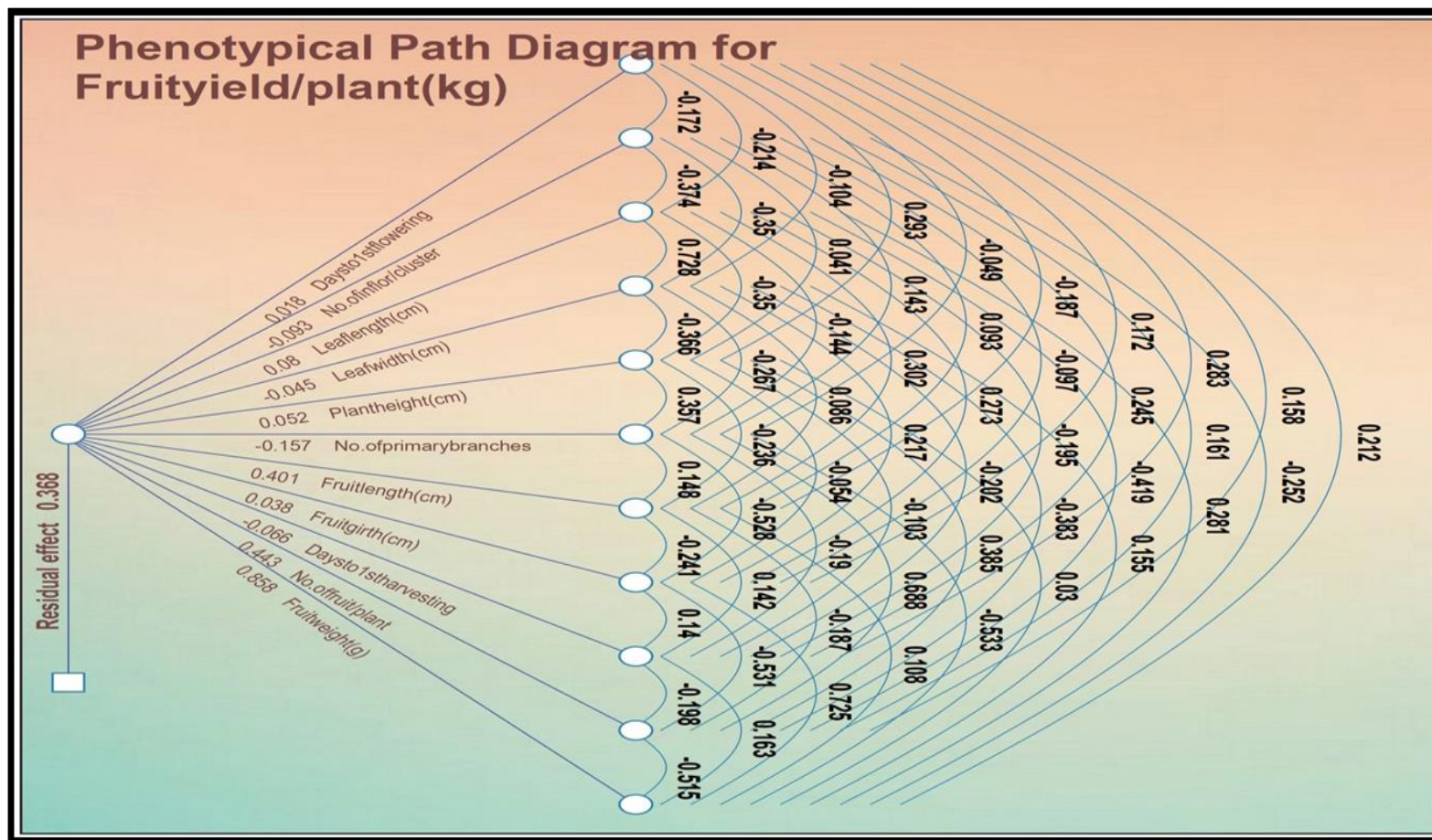


Figure 4.1.8 Direct (diagonal) and indirect effects of morphological characters on fruit yield/plant at phenotypic level in brinjal genotypes

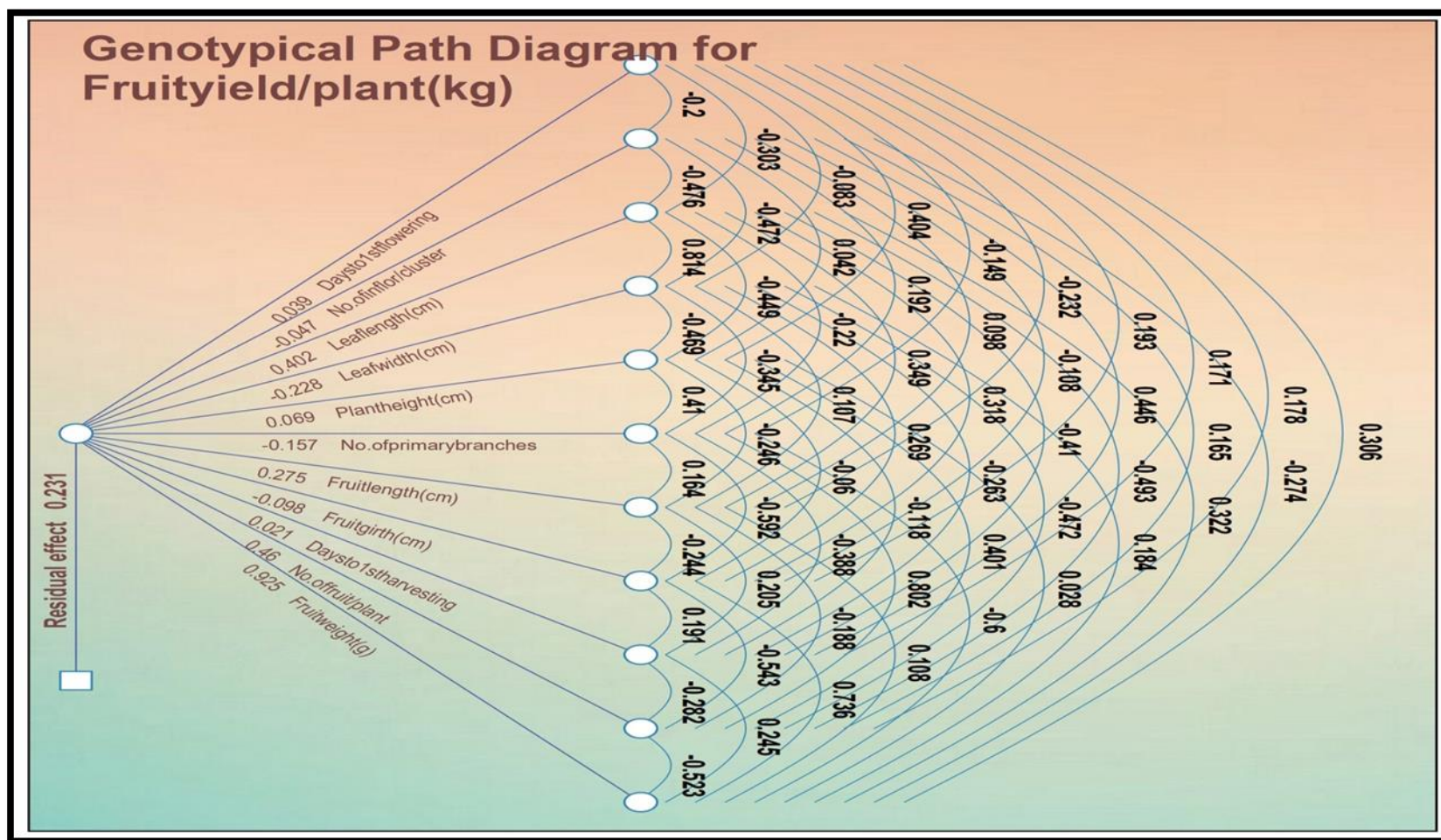


Figure 4.1.9 Direct (diagonal) and indirect effects of morphological characters on fruit yield /plant at genotypic level in brinjal genotypes

4.1.2.5 Genetic divergence of different morphological characters

4.1.2.5a Mahalanobis generalized distance (D^2)

The genetic diversity among twenty two genotypes was measured by applying D^2 statistics (Mahalanobis). Out of twelve (12) characters studied, number of fruits/plant contributed maximum percent towards genetic divergence (62.77%) followed by fruit length (11.26%), fruit weight (9.96%), fruit girth (7.79%), fruit yield/plant (3.90%), plant height (2.16%), number of flowers/inflorescence (1.73%) and days to 1st harvesting (0.430%) contributes with same percentage in Table 4.1.8 and Figure 4.1.10 respectively.

Table 4.1.8 Maximum percentage contributions of characters towards diversity in brinjal genotypes

Source	Character	Contribution (%)	Times Ranked first
1	No. of fruits/plant	62.77	145
2	Fruit length (cm)	11.26	26
3	Fruit weight (g)	9.96	23
4	Fruit girth (cm)	7.79	18
5	Fruit yield/plant (kg)	3.90	9
6	Plant height (cm)	2.16	5
7	No. of flowers/inflorescence	1.73	4
8	Days to 1st harvesting	0.43	1

As per Tocher's method (Rao, 1952) has grouped into five clusters which was based on D^2 values Figure 4.1.11. The D^2 statistics which is the sum of square for each Y values was calculated for all combinations. Cluster I was largest group comprising of 17 genotypes, followed by cluster III with 2 genotypes, cluster II, cluster IV and cluster V were containing single genotype each (Table 4.1.9). D^2 value ranged from 65.01 to 1538.29 among genotypes (Table 4.1.8).

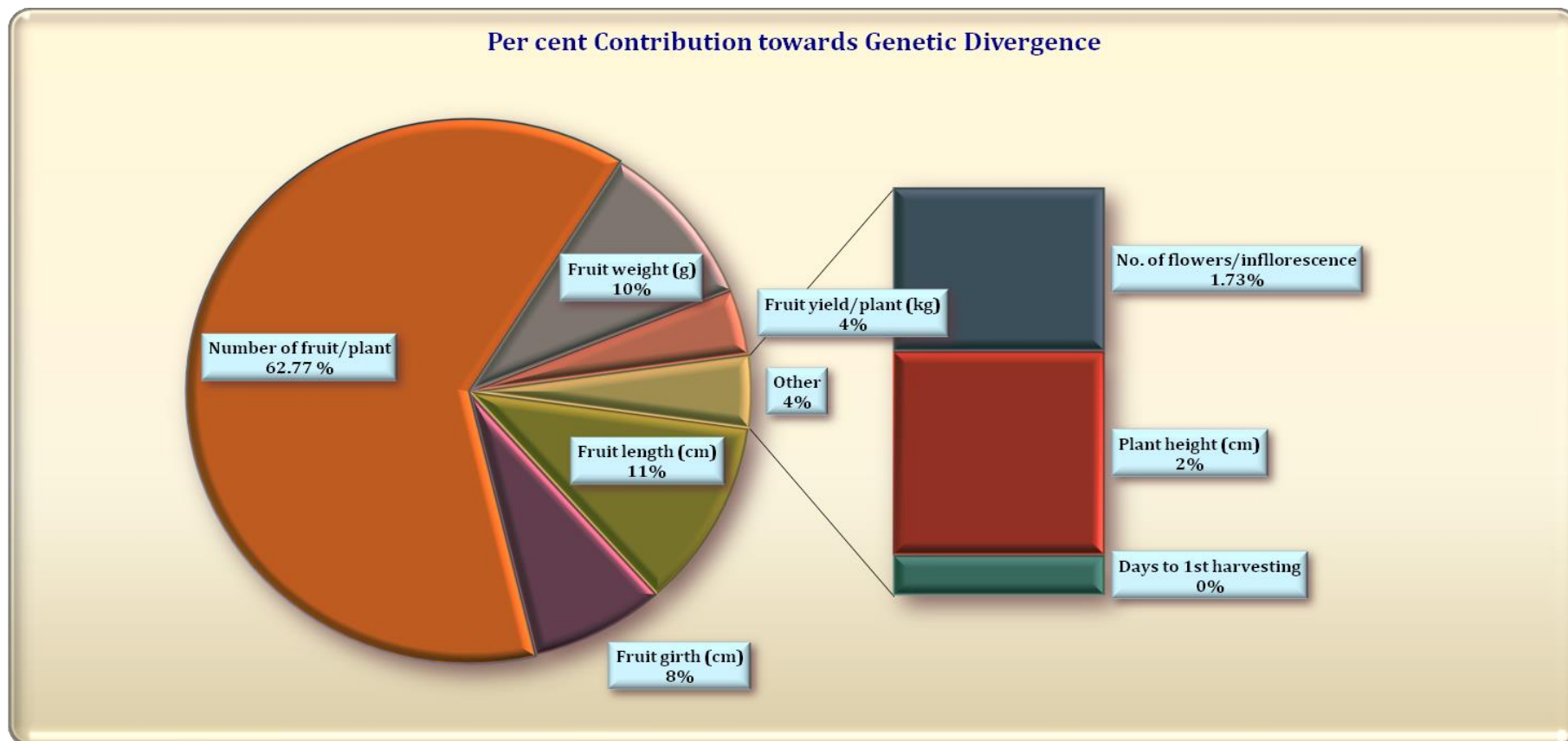


Figure 4.1.10 Contribution % towards genetic divergences

Table 4.1.9 Distribution of clusters and their respective genotypes by Tocher's method

Cluster	Number of genotypes	Genotypes
1 Cluster	17	CHFB-19, CHFB-20, CHFB-21, CHFB-22, CHFB-23, CHFB-24, CHFB-27, CHFB-28, CHFB-29, CHFB-30, CHFB-32, CHFB-34, CHFB-35, CHFB-36, CHFB-3, Arka Nidhi and Arka Keshav
2 Cluster	1	CHFB-18
3 Cluster	2	CHFB-25, CHFB-26
4 Cluster	1	CHFB-31
5 Cluster	1	CHFB-33

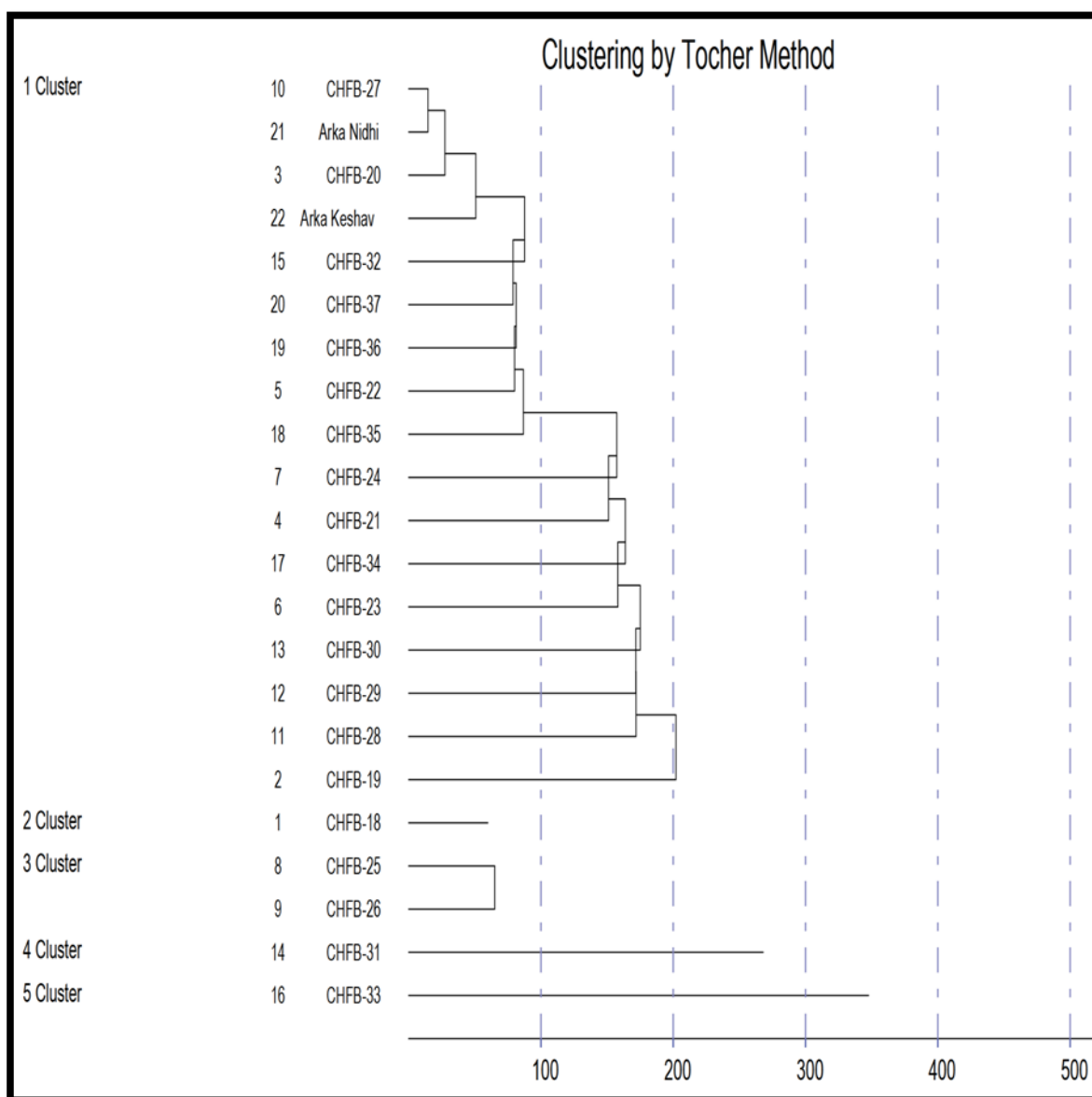


Figure 4.1.11 Dendrogram based on morphometric character of twenty two genotypes of brinjal for twelve traits using hierarchical cluster analysis (Tocher's method)

4.1.2.5b Cluster distance

The intra and inter cluster distance were observed which represented that index of genetic diversity among the traits as shown in the Table 4.1.10. The greater intra cluster distance showed that there is maximum considerable amount of genetic diversity among the genotypes. The maximum intra cluster distance was observed in cluster I carrying 17 genotypes showed maximum intra cluster diversity ($D^2 = 159.36$) while cluster III had two genotypes showing diversity with minimum value ($D^2 = 65.01$). Cluster II, IV, and V had no intra cluster ($D^2 = 0.00$) as they possessed single genotypes genetically varying from each other. In relation to inter cluster distance, cluster III recorded greater divergence with cluster V which indicated that the members of these two clusters are genetically very diverse to each other. The genotypes observed under this group could be applied for crop improvement through intercrossing. Minimum inter cluster distance was observed between cluster I and cluster II (385.17) which indicates close relationship between genotypes.

Higher degree of divergence among the genotypes within a cluster could produce more segregating breeding materials after hybridization, even selection within such cluster can be done based on maximum mean value for the desirable traits. Inter cluster distance of the genotypes were observed based on distance between the cluster and maximum inter cluster distance was observed between cluster III and cluster IV ($D^2 = 1538.29$) followed by cluster II and cluster V ($D^2 = 1057.25$), cluster II and cluster IV ($D^2 = 791.34$), cluster II and cluster III ($D^2 = 756.87$) and cluster II and cluster IV ($D^2 = 720.71$) indicated, the members of these two clusters are genetically very diverse to each other. The minimum inter cluster distance recorded between cluster I and II ($D^2 = 385.17$) indicated that the genotypes of the clusters were not much genetically diverse from each other. Hence these genotypes can be used singly as well as multiple crossing programme for development of hybrid and segregating population.

4.1.2.5c Cluster mean analysis of different morphological characters

As the character wise mean performance of the clusters observed separately Table 4.1.11. days to 1st flowering observed maximum in the cluster II followed by cluster V, cluster IV, I and cluster III while in case of number of flowers per inflorescence, maximum was found in cluster V followed by cluster III, cluster IV and

cluster I, where cluster II recorded minimum. Highest cluster means (17.8) for leaf length was observed in the cluster III followed by cluster II, cluster I and cluster IV where lowest was in the cluster V (7.97). The cluster means for leaf width was recorded maximum in the cluster II (11.08) followed by cluster I, cluster III, cluster IV and cluster V. The genotypes in cluster V having highest mean value for plant height (197.33) followed by cluster IV, cluster III, cluster II and cluster I. Number of primary branches observed higher value in the cluster IV (10.33) followed by cluster III, cluster V, and cluster I, where cluster II was recorded low (4.67). The genotypes of the cluster III had maximum fruit length followed by cluster I, cluster II and cluster IV while least value appeared in cluster V (1.20). The maximum value of fruit girth was observed in cluster II (8.95), where cluster V showed least value (1.22). The mean of cluster I, III and cluster IV observed 4.60 cm, 2.38cm and 1.37cm respectively. Days to 1st harvesting recorded maximum in cluster V (74.33) followed by cluster I, cluster II cluster III and cluster IV. Highest mean value was registered for number of fruits per plant (33.33) while, cluster V found lower mean value than cluster IV followed by cluster III and cluster I. The least mean value was found in cluster II for the trait number of fruits per plant. In case of fruit weight, cluster II registered highest (507.42g) mean value, while cluster V recorded lowest fruit weight (3.12g). Average fruit weight mean values were registered for cluster III, I and cluster IV respectively. The maximum mean for fruit yield per plant was noted in cluster II (2.53kg) followed by cluster III, IV and I. While, least mean was observed in cluster V (0.05g). On the basis of above findings, It showed that, genotypes collected and taken under investigation have wide diversity and suitable for intercrossing.

Table 4.1.10 Average cluster distances (D^2) for twenty two genotypes of brinjal

Cluster Distances					
	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
Cluster 1	159.36	385.17	402.72	411.77	667.15
Cluster 2		0.00	756.87	791.34	1057.25
Cluster 3			65.01	720.71	1538.29
Cluster 4				0.00	401.04
Cluster 5					0

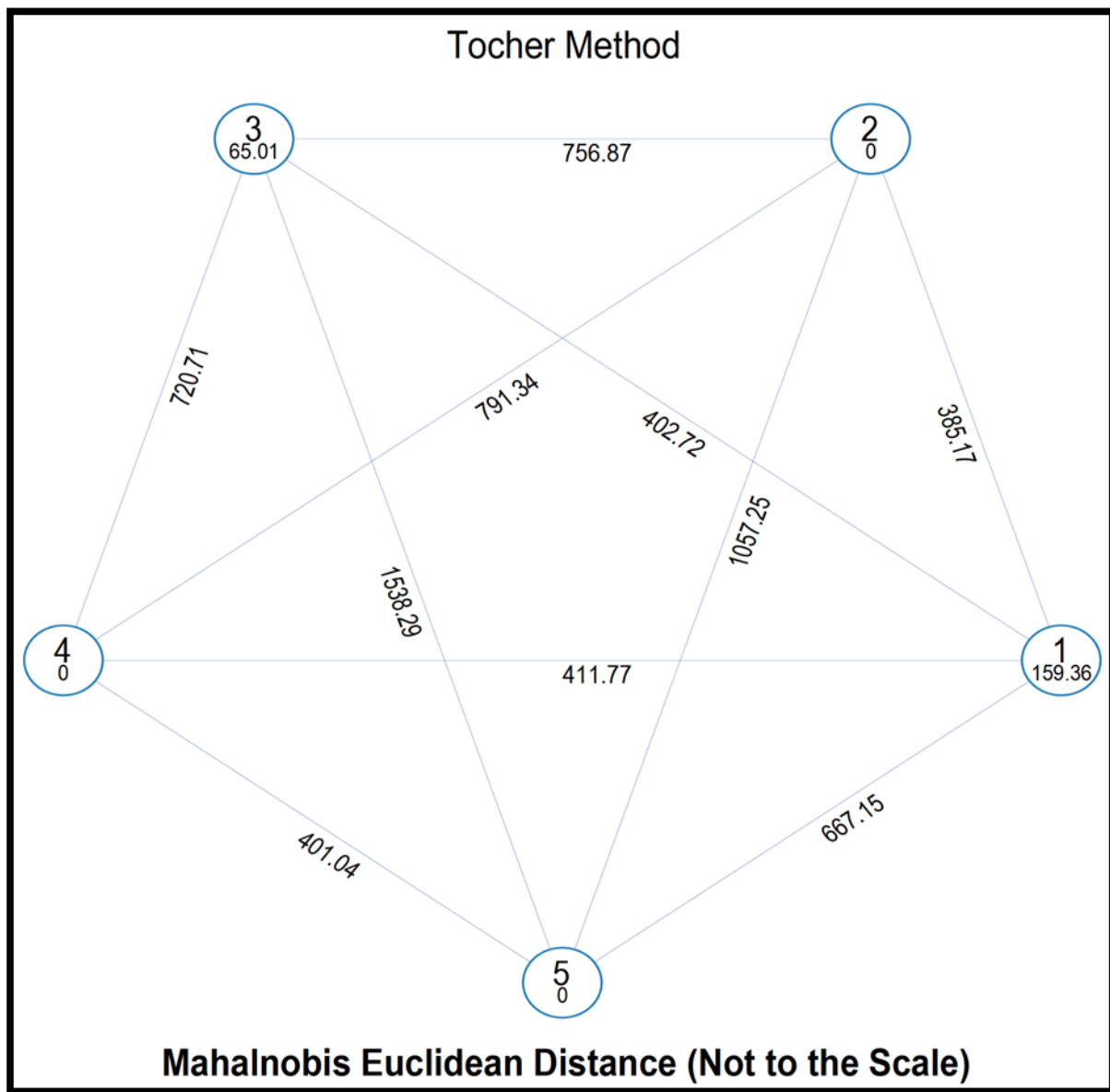


Figure 4.1.12 Cluster diagram with inter and intra distance (Not to the scale)

Table 4.1.11 Cluster Means analysis

	Days to 1st flowering	No. of flowers/inf lorescence	Leaf length (cm)	Leaf width (cm)	Plant height (cm)	No. of primary branches	Fruit length (cm)	Fruit girth (cm)	Days to 1st harvesting	No. of fruits/plant	Fruit weight (g)	Fruit yield/plant (kg)
Cluster 1	55.22	2.75	16.43	10.87	109.75	6.37	9.65	4.6	73.71	7.61	155.77	1.1
Cluster 2	60.5	2	17.77	11.08	134.67	4.67	8.5	8.85	72	4.67	507.42	2.53
Cluster 3	53.98	3	17.8	9.49	139.17	8.33	17.5	2.38	71.5	10.33	162.96	1.77
Cluster 4	58.14	3	13.27	7.51	165	10.33	8	1.37	67	33.33	46.72	1.35
Cluster 5	58.61	4	7.97	5.69	197.33	7.67	1.2	1.22	74.33	18	3.12	0.05

4.2 Screening of brinjal genotypes against bacterial wilt in field condition

Bacterial wilt caused by *Ralstonia solanacearum* in brinjal is the most devastating problem for successful cultivation in north eastern region of India. For successful cultivation of brinjal in infected areas, the only way is to use resistant varieties or to find out land races which are resistant to this disease. In the present study, the yield and yield related characteristics which showed resistance are available in these genotypes. Twenty genotypes of brinjal were collected from north eastern region of India along with two checks and grown in sick soil which was also inoculated with bacterial culture to screen out the resistant genotypes against bacterial wilt disease. The objectives of screening against bacterial wilt were recorded as follows:

4.2.1 Days to 1st incidence of bacterial wilt symptoms after inoculation

As per the Table 4.2.1, the early effect was seen by 6 days after inoculation of pathogen in the genotype CHFB- 36, followed by CHFB- 27, CHFB-32 and CHFB- 37 after 7 days of inoculation while CHFB- 33 showed late symptoms *i.e.*, 15 days after inoculation.

4.2.2 Disease severity index (%)

In case of disease severity index, the minimum disease severity was recorded in CHFB-22, CHFB-29 and CHFB-33 (20%) which indicated highly resistant against bacterial wilt. Severity index of this disease was maximum in CHFB-36 (84%) followed by CHFB-32 (76%), CHFB-27 (68%) and CHFB-37 (68%) showed susceptible. Disease severity index showed nil in the genotypes Arka Nidhi and Arka Keshav used as checks under study.

Based on rating scale against bacterial wilt of brinjal (0-5) by Winstead and Kelman (1952), 3 genotypes *i.e.*, CHFB-22, CHFB-29, CHFB-33 grouped in one category showed resistant among these twenty two genotypes, 9 genotypes such as CHFB-21, CHFB-23, CHFB-24, CHFB-26, CHFB-28, CHFB-30, CHFB-31, CHFB-34 and CHFB-35 grouped in moderately resistant, 4 genotypes CHFB-20, CHFB-25, CHFB-18 and CHFB-19 grouped in moderately susceptible, 3 genotypes CHFB-27, CHFB-32 and CHFB-37 grouped in susceptible and 1 genotype CHFB-36 recorded highly susceptible at field level.

The lines CHFB-22, CHFB-29 and CHFB-33 observed resistant in field condition, can be used in breeding programme for development improved lines through hybridization.

4.2.3. Plant disease incidence (%)

Plant Disease Incidence was recorded at 1-15, 15-25, 25-30, 30-50 and 50-60 days after inoculation. CHFB-36 (83.33%) recorded maximum disease incidence at 50-60 days after inoculation followed by CHFB- 27, CHFB-32 and CHFB-37 (72.22%). While, CHFB-22, CHFB-29 (20.00%) recorded minimum disease incidence followed by CHFB-21, CHFB-23, CHFB-26 and CHFB-34 (33.33%). No incidence was seen in Arka Nidhi and Arka Keshav.

4.2.4. Cluster analysis

Clustering of all twenty two brinjal genotypes were done on the basis of its morphological traits against bacterial wilt and all the genotypes were grouped in two major clusters (I and II) (Table 4.2.4). Major cluster I was further divided into two sub clusters (IA and IB) and Major cluster II was also further divided into two sub clusters (IIA and IIB). Arka Nidhi, Arka Keshav were grouped in sub cluster IA and showed highly resistant. Sub cluster IB had maximum genotypes CHFB-20, CHFB-25, CHFB-18, CHFB-19, CHFB-22, CHFB-29, CHFB-33, CHFB-26, CHFB-34, CHFB-21, CHFB-24, CHFB-28, CHFB-31, CHFB-35, CHFB-30, CHFB-23 observed resistant, moderately resistant and moderately susceptible. CHFB- 27, 32 and CHFB- 37 recorded susceptible under sub cluster IIA. Genotype CHFB- 36 was observed highly susceptible and grouped under sub cluster IIB. These grouping are also confirmed with the dendrogram of hierarchical cluster analysis which showed average linkage between the groups.

Table 4.2.1 Screening against bacterial wilt in field condition

Genotypes	Days to 1st incidence of bacterial wilt symptoms after inoculation	Disease Severity Index (%)
CHFB-18	9	56.00
CHFB-19	8	60.00
CHFB-20	9	52.00
CHFB-21	11	40.00
CHFB-22	14	20.00
CHFB-23	12	36.00
CHFB-24	10	40.00
CHFB-25	9	52.00
CHFB-26	12	32.00
CHFB-27	7	68.00
CHFB-28	12	40.00
CHFB-29	14	20.00
CHFB-30	11	40.00
CHFB-31	11	40.00
CHFB-32	7	76.00
CHFB-33	15	20.00
CHFB-34	11	36.00
CHFB-35	12	40.00
CHFB-36	6	84.00
CHFB-37	7	68.00
ARKA NIDHI	-	0.00
ARKA KESHAV	-	0.00

Table 4.2.2 Plant disease incidence (%) of twenty two brinjal genotypes

Genotypes	1-15 DAI	15-25 DAI	25-30 DAI	30-50 DAI	50-60 DAI
CHFB-18	16.67	20.00	33.33	44.44	61.11
CHFB-19	16.67	27.78	33.33	50.00	66.66
CHFB-20	11.11	16.67	20.00	44.44	50.00
CHFB-21	11.11	20.00	27.78	33.33	33.33
CHFB-22	5.55	5.55	11.11	16.67	20.00
CHFB-23	16.67	27.78	33.33	33.33	33.33
CHFB-24	11.11	16.67	16.67	27.78	27.78
CHFB-25	11.11	22.22	22.22	38.89	44.44
CHFB-26	11.11	20.00	22.22	27.78	33.33
CHFB-27	16.22	33.34	44.44	61.11	72.22
CHFB-28	11.11	16.67	16.67	27.78	27.78
CHFB-29	5.55	11.11	16.67	16.67	20.00
CHFB-30	16.67	16.67	16.67	22.22	22.22
CHFB-31	16.67	16.67	20.00	27.78	27.78
CHFB-32	27.78	44.44	50.00	61.11	72.22
CHFB-33	0.00	5.55	11.11	11.11	16.67
CHFB-34	11.11	16.67	22.22	27.78	33.33
CHFB-35	11.11	11.11	16.67	22.22	27.78
CHFB-36	33.33	55.55	61.11	83.33	83.33
CHFB-37	27.78	33.33	44.44	61.11	72.22
Arka Nidhi	0.00	0.00	0.00	0.00	0.00
Arka Keshav	0.00	0.00	0.00	0.00	0.00

Table 4.2.3 Based on the rating scale against bacterial wilt of brinjal (0-5) by Winstead and Kelman (1952)

Severity of disease	No. of genotypes	Genotypes
Highly resistant/ Immune	2	Arka Nidhi, Arka Keshav
Resistant	3	CHFB- 22 , CHFB-29, CHFB-33
Moderately resistant	9	CHFB- 21 , CHFB-23 , CHFB-24 , CHFB- 26 , CHFB-28, CHFB- 30 , CHFB-31 , CHFB- 34, CHFB-35
Moderately susceptible	4	CHFB-20, CHFB-25, CHFB-18 , CHFB-19
Susceptible	3	CHFB-27 , CHFB-32, CHFB-37
Highly Susceptible	1	CHFB-36

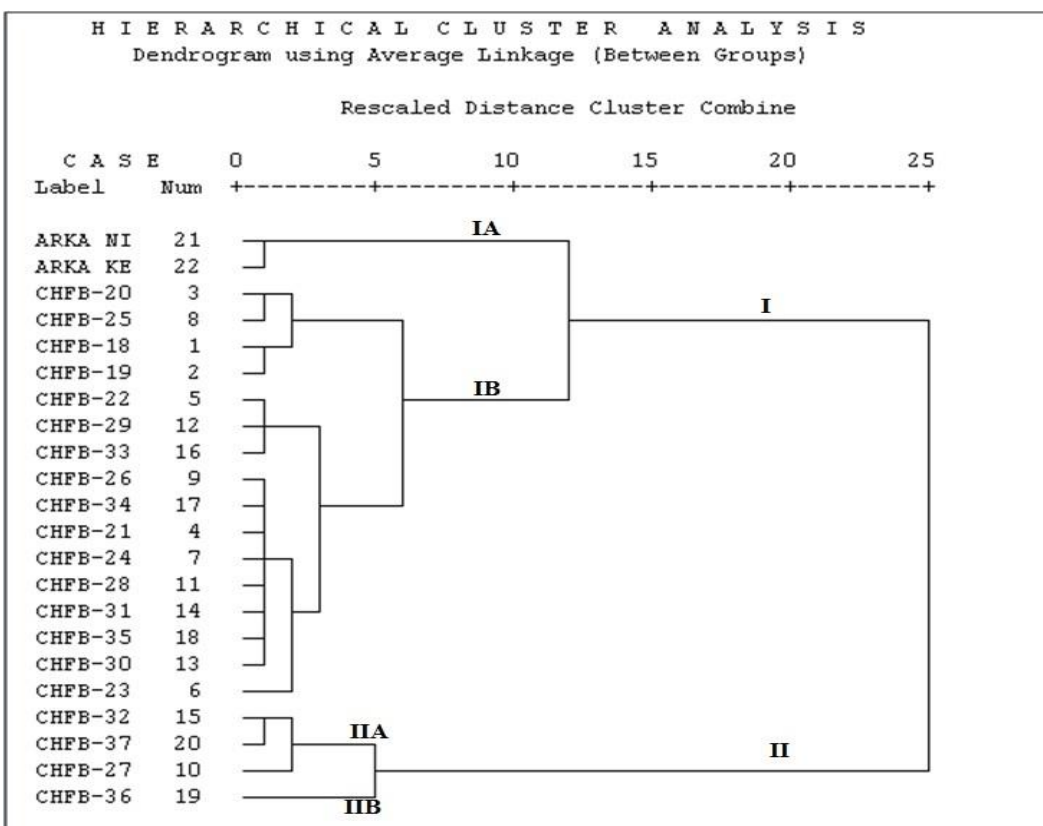


Figure 4.2.1 Dendrogram of morphological characters of twenty two brinjal genotypes against bacterial wilt

Table 4.2.4 Clustering of morphological characters for twenty two brinjal genotypes against bacterial wilt

Major Cluster (2)	Sub cluster	Genotypes
I	IA	Arka Nidhi, Arka Keshav
	IB	CHFB-20, CHFB-25, CHFB-18, CHFB-19, CHFB-22, CHFB-29, CHFB-33, CHFB-26, CHFB-34, CHFB-21, CHFB-24, CHFB-28, CHFB-31, CHFB-35, CHFB-30, CHFB-23
II	IIA	CHFB-27, CHFB-32, CHFB-37
	IIB	CHFB-36

4.3 Assessment of bacterial wilt resistant brinjal genotypes based on molecular analysis

4.3.1 DNA isolation and purity assessment

The genomic DNA was isolated from 22 brinjal genotypes which yielded good amount of DNA. The quality and quantity of isolated DNA samples were tested and found good by agarose gel electrophoresis and spectrophotometer respectively. The DNA quantity for all the genotypes is presented in Table 4.3.1. The quantity of DNA from different samples varied from 273 ng/μl (CHFB-29) to 1200 ng/μl (CHFB-22).

Table 4.3.1 Genomic DNA quantity in twenty two brinjal genotypes

Sl. No.	Cultivars	OD ₂₆₀ / OD ₂₈₀	DNA content (ng/μl)
1.	CHFB-18	1.55	675
2.	CHFB-19	1.60	738
3.	CHFB-20	1.75	342
4.	CHFB-21	1.70	609
5.	CHFB-22	1.48	1200
6.	CHFB-23	1.63	642
7.	CHFB-24	1.72	672
8.	CHFB-25	1.73	312
9.	CHFB-26	1.42	720
10.	CHFB-27	1.89	363
11.	CHFB-28	1.62	681
12.	CHFB-29	2.00	273
13.	CHFB-30	1.63	942
14.	CHFB-31	1.60	342
15.	CHFB-32	1.75	495
16.	CHFB-33	1.78	933
17.	CHFB-34	1.60	810
18.	CHFB-35	1.59	876
19.	CHFB-36	1.73	373
20.	CHFB-37	1.58	537
21.	Arka Nidhi	1.59	732
22.	Arka Keshav	1.68	585

4.3.2 Optimization of PCR protocol

4.3.2.1 Primer selection for SSR analysis

After screening 20 primer pairs, a set of 13 consistent primer pairs that produced clearly distinguishable DNA banding patterns, were selected for analysis of molecular characterization and diversity studies.

4.3.2.2 Generation of SSR profiles for molecular characterization

A total of 20 SSR loci were used to analyze genetic diversity among 22 brinjal genotypes, and 40 alleles with allele size ranging from 50 to 320 bp were detected with an average of 3.07 alleles per locus. Number of bands amplified by 13 primers ranged from 2 to 6 alleles. Out of 20 SSR loci, 13 were polymorphic showing 87.82 per cent of polymorphism among 22 genotypes. Among 13 SSR primer pairs, four primer pairs emi04H10, emg11I04, emb01E03, and emf21E10 amplified two alleles; six primer pairs EEMS46, SSR 1, emf21K08, eme25D01, emg11A06 and emf01D24 amplified three alleles; SSR 2 and emb01D10 amplified four alleles; emb01J19 amplified six alleles (Table 4.3.2). All 22 genotypes were unequivocally differentiated using 20 SSR loci. SSR fingerprints/gel profiles generated for 22 brinjal genotypes using different primers pairs are illustrated in (Figure 4.3.1 and Figure 4.3.2).

Table 4.3.2 Details of SSR primers used for molecular characterization of twenty two brinjal genotypes

Sl. No.	Primer code	Microsatellite primer sequence (5'→3')	Observed Allele Length Range (bp)	Annealing temperature (°C)	Total bands produced (No.)	Polymorphic bands (No.)	Polymorphism (%)
1.	EEMS46	F: ACCAAACGTGCATGAAACAA R: GGAAATGTTGGTGAATTGG	50-220	48	3	3	100.00
2.	SSR 1	F: GTGACTACGGTTTCACTGGT R: GATGACGACGACGATAATAGA	50-260	50	3	3	100.00
3.	SSR 2	F: ATTGAAAGTTGCTCTGCTTC R: GATCGAACCCACATCATC	50-230	48	4	4	100.00
4.	emf21K08	F: ATCAATGACACCCAAAACCCATTT R: GTTTGAAAACCCAATACAAATCCGA	150-240	54	3	2	66.67
5.	emi04H10	F: ATCGGAGCAAGAGACATTAGATGC R: GTTTCCAACACAGTCCCCAATACAACAA	100-240	50	2	2	100.00
6.	emb01D10	F: AAGAATCGGTCCTCTTTGCATTGT R: TGCTTTTCACCTCTCCGCTATCTC	50-240	50	4	3	75.00
7.	emb01J19	F: GACAGGGATAGGGGTACGGATAGG R: ATCCATGTGATGCCTCGATTTTCT	50-280	55	6	5	83.33
8.	eme25D01	F: AGTCCCAACCAAAATCGTAGAGGC R: GTTTCACTGAAGGATGTGGAGTGTGA	100-300	54	3	2	66.67
9.	emg11I04	F: ACATTCAGGTTTCTCTCTTCCCCTC R: GTTTGCCCTCCACTCTTGATCATCTTT	50-80	555	2	2	100.00
10.	emg11A06	F: AGTGCTAATATGCAAGGGGAATGG R: GTTTACGGTGATCTTTCCGTATTCCAAA	70-280	555	3	3	100.00
11.	emb01E03	F: GCGAGAATTTAAAAGGGGGAAGTG R: TTGAACCGTCAAGATCCTTCCATT	300-320	55	2	2	100.00
12.	emf21E10	F: AAGAGAGTCGAGAGAGGTGGATTG R: GTTTCTCCTTTGCAATCTCTTTTCA	50-90	53	2	1	50.00
13.	emf01D24	F: ATACAGTGCCCAACACGATTCAAG R: GTTTCAGATAGATGGAATTAAGGGGGTG	90-260	55	3	3	100.00
	Total				40	35	87.82
	Average				3.07	1.75	

4.3.3 Genetic diversity parameters for SSR primers

Among 22 brinjal genotypes, SSR primer pairs exhibited higher level of polymorphism. The different band statistics and efficiency parameters calculated is given below:

Table 4.3.3 Molecular diversity parameters related to efficiency of 13 SSR markers

Sl. No.	Marker Name	Allele/ marker	PIC * value	MI*	DI*	RP*
1.	EEMS46	3	0.58	17.55	0.86	1.80
2.	SSR 1	3	0.44	13.38	0.81	1.84
3.	SSR 2	4	0.75	30.19	0.93	1.45
4.	emf21K08	3	0.52	15.62	0.84	1.82
5.	emi04H10	2	0.49	9.95	0.74	1.86
6.	emb01D10	4	0.60	24.22	0.90	2.00
7.	emb01J19	6	0.70	42.28	0.95	2.00
8.	eme25D01	3	0.62	18.77	0.87	2.00
9.	emg11I04	2	0.48	9.66	0.74	1.63
10.	emg11A06	3	0.60	18.11	0.86	2.00
11.	emb01E03	2	0.25	5.10	0.62	0.60
12.	emf21E10	2	0.50	10.00	0.75	2.00
13.	emf01D24	3	0.52	15.62	0.84	1.53
	Average	3.07	0.54	17.73	0.82	1.73

*PIC-Polymorphic Information Content, *MI-Marker Index, *DI-Diversity Index, *RP- Resolving Power

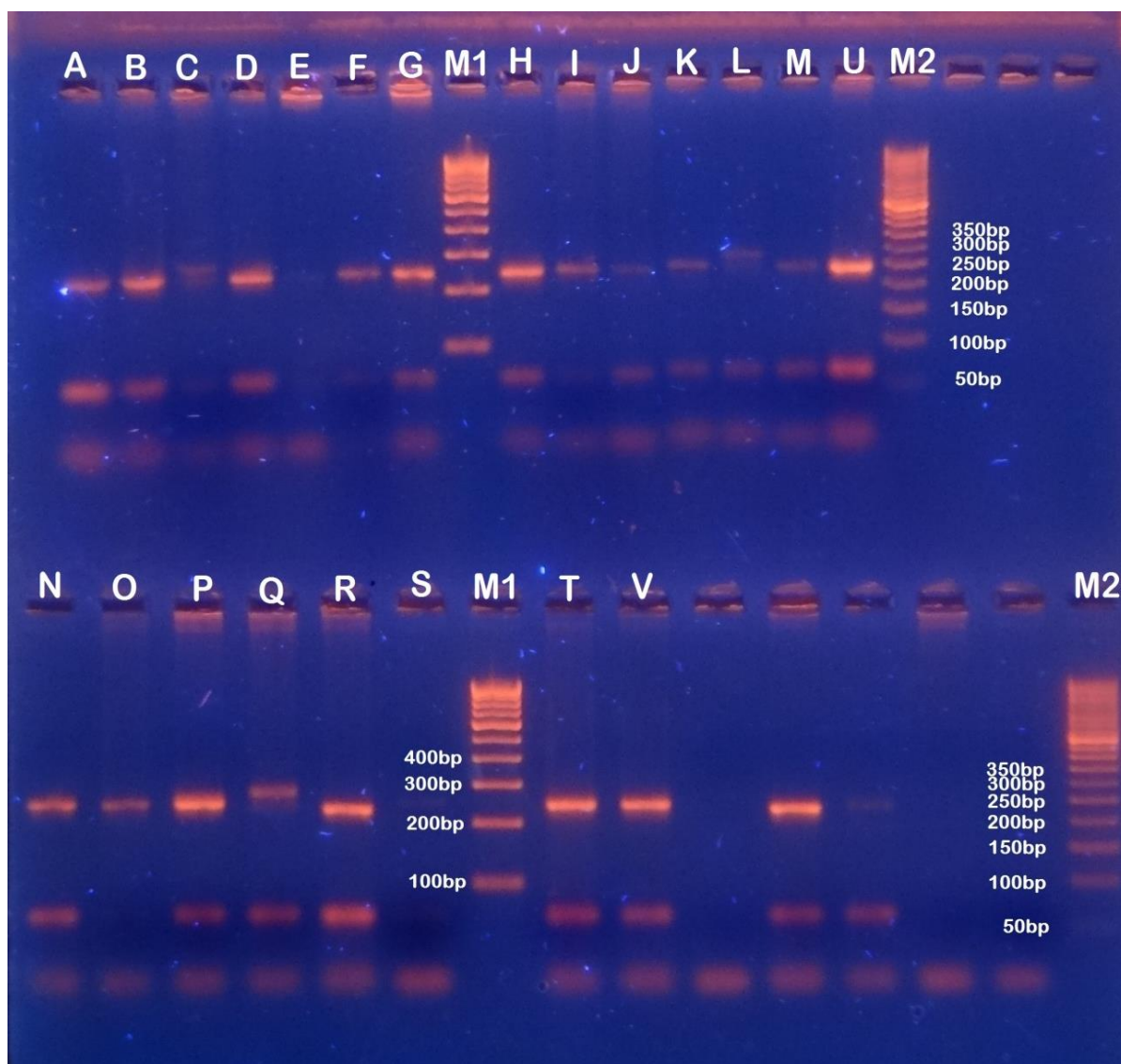


Figure 4.3.1 SSR profiling of twenty two brinjal genotypes using primer emb01D10

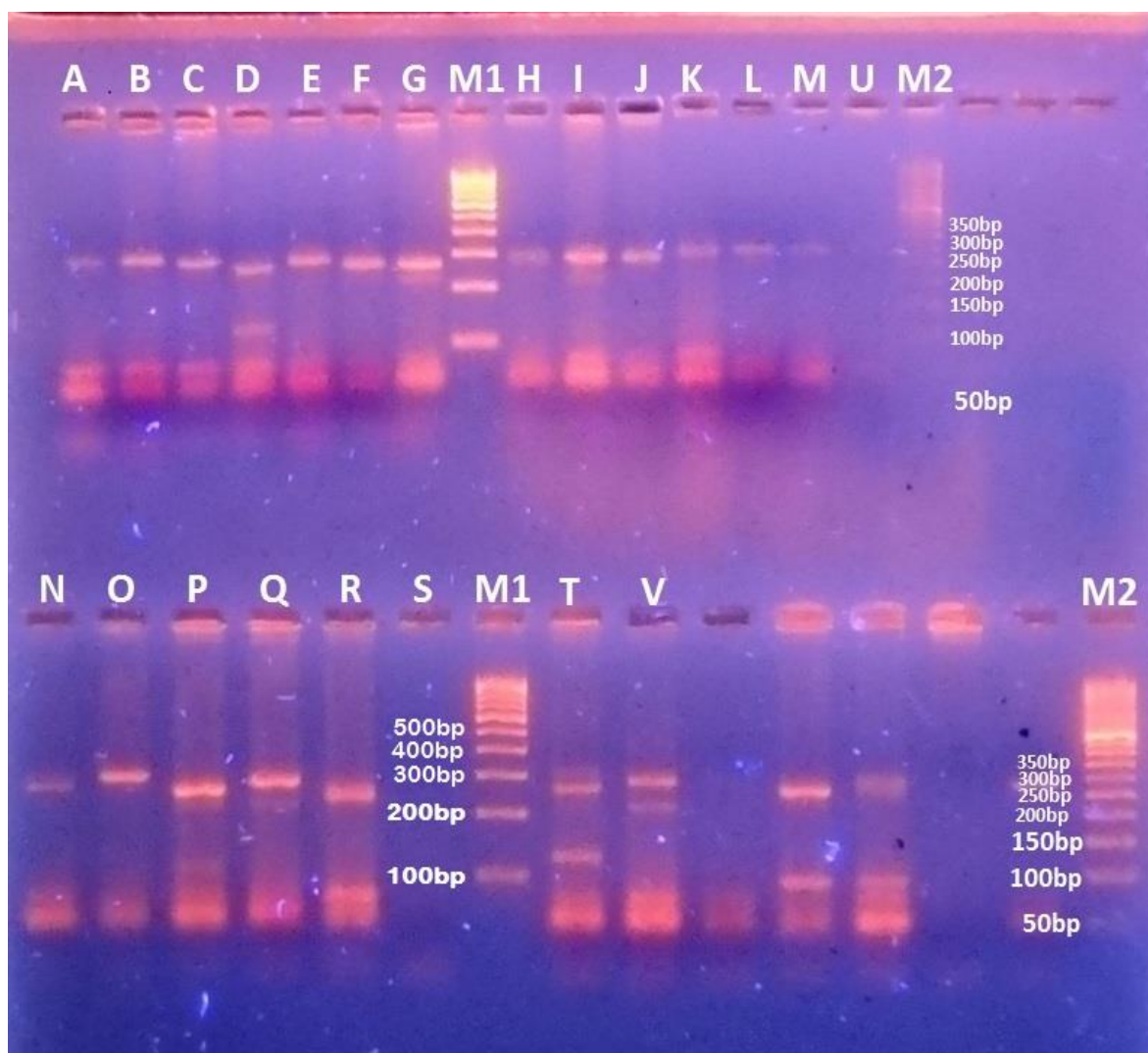


Figure 4.3.2 SSR profiling of twenty two brinjal genotypes using primer emb01J19 where, M₁: 100bp Ladder; M₂: 50 bp Ladder and A-V are the brinjal genotypes

A= CHFB-18, B= CHFB-19, C= CHFB-20, D= CHFB-21, E= CHFB-22, F= CHFB-23, G= CHFB-24, H= CHFB-25, I= CHFB-26, J= CHFB-27, K= CHFB-28, L= CHFB-29, M= CHFB-30, N= CHFB-31, O= CHFB-32, P= CHFB-33, Q= CHFB-34, R= CHFB-35, S= CHFB-36, T= CHFB-37, U= Arka Nidhi, V= Arka Keshav

4.3.3.1 Polymorphic information content (PIC)

Among genotypes, the polymorphic information content (PIC) value ranged from 0.25 to 0.75 with an average of 0.54 for different primer pairs. The maximum PIC value (0.75) was found in primer SSR 2 followed by emb01J19 primer (0.70). The least PIC value was found in primer emb01E03 (0.25), followed by primer SSR 1 (0.44).

4.3.3.2 Marker Index (MI)

The marker index (MI) value ranged from 5.1 to 42.28 with a mean of 17.73. The maximum value for marker index (MI) was observed for the primer emb01J19 (42.28) followed by primer SSR 2 (30.19) and primer emb01D10 (24.22) while, lowest value for primer was emb01E03 (5.10) followed by primer emg11I04 (9.66), primer emi04H10 (9.95).

4.3.3.3 Diversity Index (DI)

The diversity index (DI) value ranged from 0.62 to 0.95 with a mean of 0.82. According to Nei's (1978), the maximum diversity index (DI) value was observed for the primer emb01J19 (0.95) followed by primer SSR 2 (0.93) and primer emb01D10 (0.90) while, lowest value was observed for primer emb01E03 (0.62) followed by primer emi04H10 (0.74) and primer emg11I04 (0.74). The higher DI value indicated that the thirteen SSR markers used in this study were informative.

4.3.3.4 Resolution Power (RP)

The resolution power (RP) value ranged from 0.60 to 2.00 with a mean of 1.73. The maximum resolution power (RP) value was observed for the primer emb01D10 (2.00), primer emb01J19 (2.00), eme25D01 (2.00), emg11A06 (2.00) and primer emf21E10 (2.00) while, lowest value was observed for primer emb01E03 (0.60) followed by primer SSR 2 (1.45).

4.3.3.5 Percentage polymorphism

Highest amount of percent polymorphism (100%) was reported by the primers EEMS46, SSR 1, SSR 2, emi04H10, emg11I04, emg11A06, emb01E03 and emf01D24 however, emf21E10 recorded the lowest (50%).

4.3.4 Cluster analysis

The Weighted Neighbor Joining dendrogram obtained by 20 SSRs distributed 22 genotypes into two major clusters, cluster I and cluster II (Figure 4.3.3).

The cluster I was further divided into two sub clusters, viz. IA (12 genotypes) and IB (8 genotypes). Sub cluster IA was further divided into sub-sub clusters IA₁ (11 genotypes) and IA₂ (1 genotype).

The sub-sub cluster IA₁ included the genotypes Arka Nidhi, Arka Keshav, CHFB-22, CHFB-26, CHFB-29, CHFB-21, CHFB-24, CHFB-23, CHFB-31, CHFB-26, CHFB-28, CHFB-30 and sub-sub cluster IA₂ included CHFB-33.

The genotypes Arka Nidhi and Arka Keshav of sub-sub cluster IA₁ are check varieties which are highly resistant against bacterial wilt. The remaining genotypes of sub-sub cluster IA₁ i.e., CHFB-22, CHFB-26, CHFB-29, CHFB-21, CHFB-24, CHFB-23, CHFB-31, CHFB-26, CHFB-28, CHFB-30 ranged from resistant to moderately susceptible grouped together with check varieties.

The genotype CHFB-33 which belongs to a different species i.e., *Solanum torvum* is observed to be resistant against bacterial wilt and is grouped under sub-sub cluster IA₂.

Sub cluster IB included genotypes CHFB-34, CHFB-35, CHFB-19, CHFB-18, CHFB-37, CHFB-20, CHFB-25, CHFB-36. These genotypes ranged from moderately resistant to moderately susceptible except for CHFB-36. Although, the genotype CHFB-36 observed most susceptible in field condition, it was grouped under major cluster I in molecular analysis. Cluster II included the genotypes CHFB-27 and CHFB-32 were found susceptible.

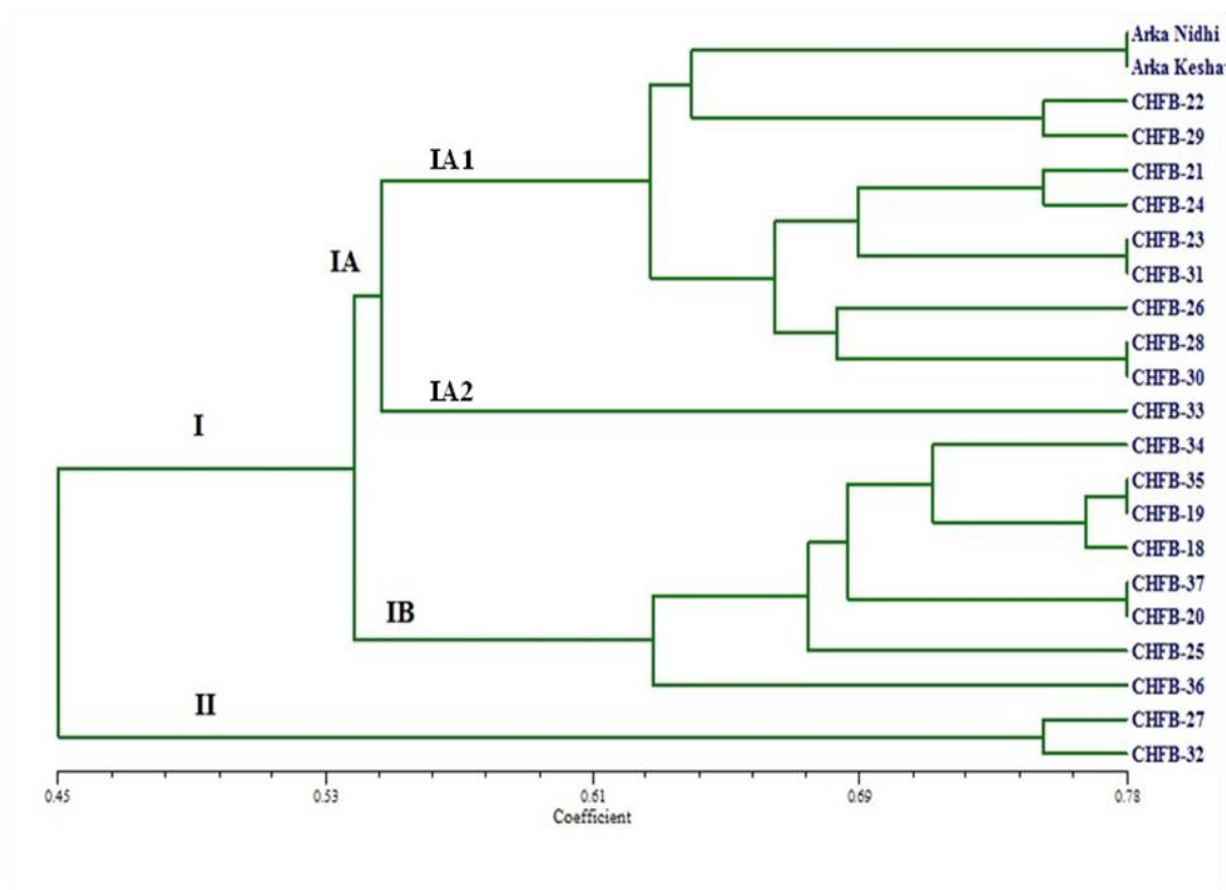


Figure 4.3.3 Dendrogram of molecular characters of twenty two brinjal genotypes

Table 4.3.4 Clustering of molecular data for twenty two brinjal genotypes

Major Cluster (2)	Sub cluster	Sub-sub cluster	Genotypes
I	IA	IA ₁	Arka Nidhi, Arka Keshav, CHFB-22, CHFB-29, CHFB-21, CHFB-24, CHFB-23, CHFB-31, CHFB-26, CHFB-28, CHFB-30
		IA ₂	CHFB-33
	IB		CHFB-34, CHFB-35, CHFB-19, CHFB-18, CHFB-37, CHFB-20, CHFB-25, CHFB-36
II			CHFB-27, CHFB-32

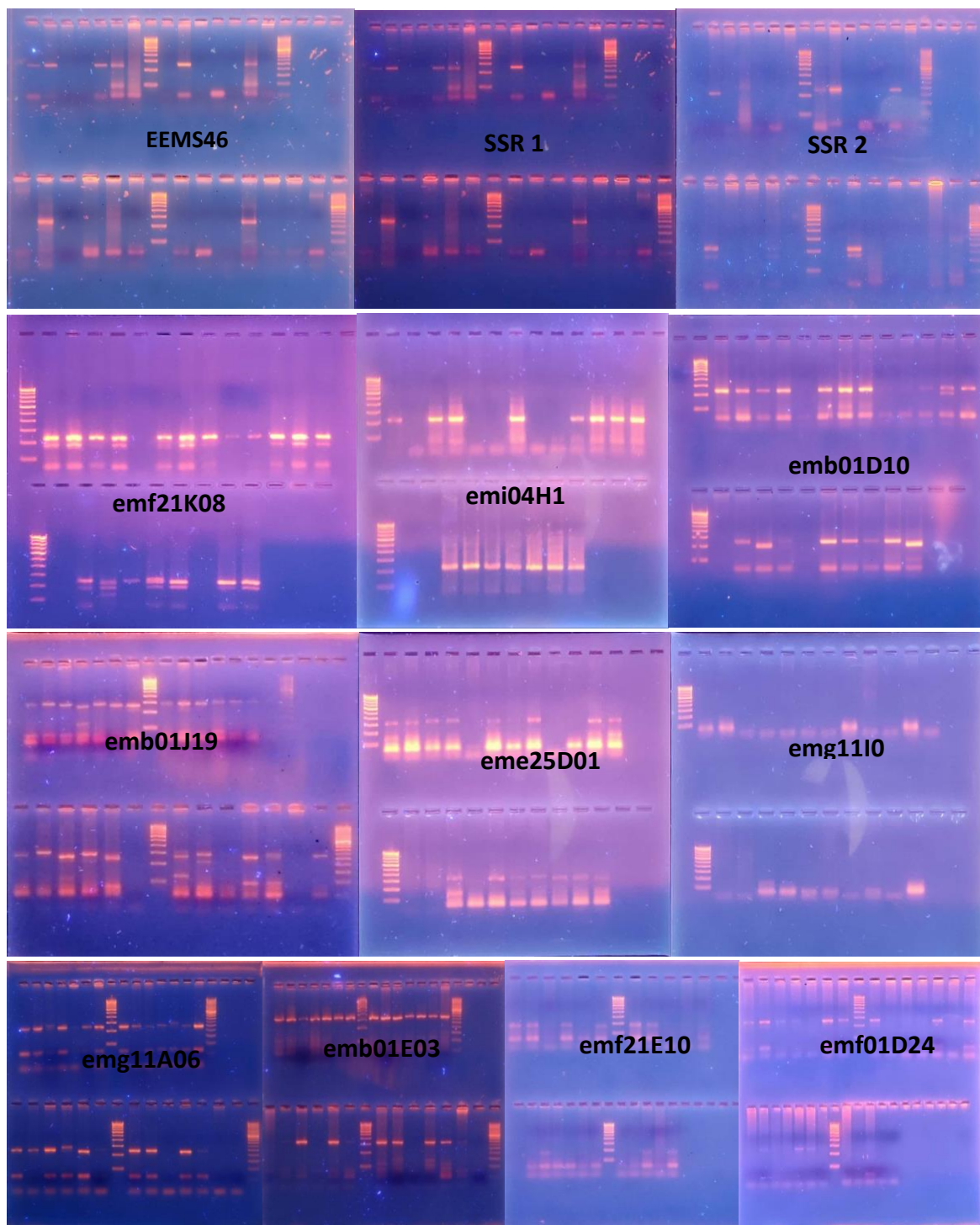


Figure 4.3.4 SSR profiling of twenty two brinjal genotypes for 13 polymorphic primers

Chapter-5

Discussion

The prime objective of most of the research programme is to make an improvement in yield, but yield is a complex character which generally depends upon all the other component traits to improve the yield. Yield is controlled poly-genetically and influenced greatly by the environmental fluctuations. The bacterial wilt disease as well as other biotic stresses is also major challenging problems which generally run side by side with crops. Selection of superior genotypes based on yield alone would not be always effective. In order to make the selection effective one has to put attention on the component characters, which contribute yield in positive direction as well as to screen out resistant source to avoid various biotic and abiotic problems especially bacterial wilt of brinjal which is the most destructive disease. In such a situation the available variability in the gene pool provides an opportunity for selecting superior genotypes, which can be obtained through screening and evaluation.

The knowledge of association between the characters and their direct and indirect contribution towards expression of characters help plant breeders in deciding the selection criteria. The identification of donor parents for important characters, assessment of existing variability present in the available germplasm and the information about character associations are required for successful breeding programmes.

To find out the resistant gene available in the present brinjal population, field observations on bacterial wilt is the conventional method to find out severity among population while, molecular markers including SSR markers are also found effective tools to screen out suitable resistant gene against bacterial wilt to use in further breeding programme and also to screen out susceptible genotypes present in population to discard for further contamination through natural crossing.

Thus, the attempt was made in the present investigation **“Screening of Brinjal Genotypes of North East India against Bacterial Wilt Using SSR Markers”**. The primary information on the magnitude of variability present in collected population were studied and also to find out resistant source against bacterial wilt through field screening and SSR markers. The findings of present investigation consisting of 22

genotypes for 12 characters, for morphological diversity, screening against bacterial wilt in field condition and molecular characterization of brinjal are discussed as follows:

5.1 Diversity Analysis

5.2 Screening of brinjal genotypes against bacterial wilt in field condition

5.3 Assessment of bacterial wilt resistant brinjal genotypes based on molecular analysis.

5.1 Diversity analysis

The assessment of existing variability in germplasm was done by computing means, range and least significant differences. The nature of association among different characters was studied by using analysis of variance by Panse and Sukhatme (1967), genetic variability (Burton and de Vane, 1953), heritability in broad sense (Hanson *et al.* 1956), correlation coefficient by Al-jibouri *et al.* (1958), path coefficient analysis by Dewey and Lu (1959), genetic divergence analysis by Mahalanobis' D^2 following Rao (1952).

5.1.1 Analysis of variance

5.1.2 Mean performance of the genotypes

5.1.3 Parameters of variability

5.1.4 Heritability and genetic advance

5.1.5 Correlation studies

5.1.6 Path coefficient analysis

5.1.7 Genetic divergence

5.1.1. Analysis of Variance

Analysis of variance indicated significant differences among genotypes for all the characters indicating that the present genotypes were appropriate and hence suitable for further genetic analysis. A wide range of variation was observed for all characters studied, particularly for days to 1st flowering, number of flowers per inflorescence, leaf length (cm), leaf width (cm), plant height (cm), number of primary branches, fruit length (cm), fruit girth (cm), days to 1st harvesting, number of fruits per

plant, fruit weight (kg), fruit yield per plant (kg). The characters showing high degree of variations have more scope for their further improvement (Mohanty, 2002).

5.1.2. Mean performance of genotypes

Significantly early flowering was observed in the genotype CHFB-36 (49.71 days) followed by genotypes CHFB-22 (50.79 days) and in the check variety Arka Keshav (50.79 days) where, genotype CHFB-37 observed late flowering 63.13 days after transplanting. A significant varietal distinction was also seen by Patel (2007) and Ansari (2010). Number of flowers per inflorescence was observed significantly higher in genotypes CHFB-33, CHFB-34, CHFB-35 and Arka Keshav. Leaf length, leaf width were observed significantly higher in CHFB-22 (20.53) and CHFB-27(13.67) respectively while least leaf length (7.97) and width (5.69) was observed in the genotype CHFB-33. Genotype CHFB-33 recorded highest plant height (197.33cm) followed by CHFB-25 (194.67 cm) showed significant variation among all the genotypes where, genotype CHFB-30 observed lowest (62.33cm). Variability in plant height was also previously reported by Singh *et al.* (2013); Patel (2007); Ram *et al.* (2007) and Mohanty and Prusti (2002) in brinjal genotypes. Number of primary branches counted significantly maximum (10.33) in CHFB-31 while minimum in CHFB-18 (4.67). A significant varietal dissimilarity was also experienced by Singh *et al.* (2013) and Ram *et al.* (2007). A great variation was observed for fruit length and fruit girth in these genotypes collected from North east states of India as well as varieties used as checks collected from IIHR, Bangalore. Minimum fruit length (1.20 cm) and girth (1.22 cm) were observed in CHFB-33 while maximum fruit length (18.00 cm) was observed in CHFB-25 followed by CHFB-26 (17.00cm) which indicates significant variation among all the genotypes. Days to first harvest observed at par in all the genotypes. Early harvesting was done 67 days after transplanting in case of genotype CHFB-31 while late harvesting (83.00 days) was done in CHFB-37 followed by CHFB-35 and CHFB-37. In support of above findings, a significant varietal contrast was also seen by Mohanty and Prusti (2002).

Number of fruits per plant and fruit weight is the major components which are responsible for increasing the yield of crops directly. Maximum number of fruits per plant (33.33) was noted in the genotype CHFB-31 which was significantly higher among all the genotypes while, minimum recorded in CHFB-18 (4.67). The genotypes collected for investigation observed significant variation for fruit weight and ranged from 3.12-507.42 (g)

where maximum fruit weight (507.42 g) in the genotype CHFB-18 while minimum fruit weight CHFB-33 (3.12 g), the fruit size was small because it belonged to a different species i.e., *Solanum torvum*. Similar observations were recorded by Kumar *et al.* (2020) in brinjal for fruit weight. Maximum fruit yield per plant was recorded (2.53 kg) in the genotypes CHFB- 18 followed by CHFB-19 (1.93 kg) which showed statistical significance among all genotypes while minimum fruit yield (0.05kg) was recorded in the genotypes CHFB-33. As the *per se* performance cannot be the sole criteria for selection programme as the value of the parent selected may not be observed in progeny. Hence, it is worthwhile to study variability parameters.

5.1.3. Coefficient of variability

The existence of genetic variability in the present population provides ample opportunities for selection being effective in the genotypes taken under study. For any sound breeding programme, it is essential to have a large variation in the materials of the hand in breeder. The characters of economic importance are generally quantitative in nature and exhibited a considerable degree of interaction with the environment. In the present investigation, high Phenotypic Coefficient of Variation (PCV) was observed for fruit weight (70.90), number of fruits per plant (66.20), fruit girth (60.16) and fruit yield per plant (47.15). Moderate PCV value was noticed for the traits leaf length while, it was recorded low for the characters days to first flowering (8.06) and days to first harvesting (8.20). The similar results of the genotypic coefficient variability of low value in correspondence with PCV were observed for all the 12 characters reported above. The results of PCV and GCV were also in conformity with the results of Sujin *et al.* (2017); Shekar *et al.* (2014); Dhaka & Soni (2012) and Kumar *et al.* (2020) in brinjal.

5.1.4. Heritability and genetic advance

The heritability along with genetic advance is more meaningful and helps in predicting the resultant effect of selection on phenotypic expression. Moderate to high heritability indicates the effectiveness with which selection of genotypes can be done on the basis of its phenotypic variation appearing in the experimental population. In the present investigation, high heritability coupled with high genetic advance were observed for the characters viz., number of flowers per cluster, leaf width, plant height, number of primary branches, fruit length, fruit girth, number of fruits per plant and fruit yield per plant. Most of these trait resultants are in accordance with the findings of Sujin *et al.* (2017);

Kumar *et al.* (2020); Bajpai *et al.* (2020) and Konyak *et al.* (2020) in brinjal while, the result of Saha *et al.* (2019), Yadav *et al.* (2014) and Prabakaran *et al.* (2013) were in support of these findings in relation to fruit per plant and fruit yield per hectare.

The result of present investigation indicated that estimates of heritability were observed very high for the entire traits except days to first harvesting. It shows that these traits will be least influenced by the environment and selection for improvements of such characters may be useful. Days to first harvesting showed average heritability which may be due to environmental effect especially low temperature which may cause delay in harvesting. The high heritability for the yield and the component characters in brinjal corroborates with the findings of Singh *et al.* (2013), Prabakaran *et al.* (2013); Kumar and Arumugam (2013); Yadav *et al.* (2014); Sujin *et al.* (2017); Kumar *et al.* (2020); Bajpai *et al.* (2020) and Konyak *et al.* (2020). Highest estimates of genetic advance as percent of mean was recorded for all the characters except days to first flowering (10.62%) and days to first harvesting (8.19%), while leaf length observed moderate (27.39%). In the agreement to the present findings, the high genetic advance for the maximum traits reported by Kumar *et al.* (2020); Bajpai *et al.* (2020), and Konyak *et al.* (2020).

5.1.5. Correlation studies

The existing relationship between yield traits are generally determined by genotypic and phenotypic correlation and based on their performance, superior characters are generally considered for selection which are directly or indirectly associated with yield. Even genotypic correlation is the genetic portion of phenotypic correlation which is heritable in nature and therefore used to orient breeding programme. Alternatively phenotypic correlation measures the degree of association of two variables and is determined by genetic and environmental factors. In the present study at the phenotypic level, fruit weight (0.818), fruit girth (0.423) exhibited highly significant positive correlation with fruit yield per plant. Fruit length (0.365), leaf length (0.282) have significant positive correlation with fruit yield per plant while, non significant positive correlation was observed in case of days to first flowering (0.210), leaf width (0.085), plant height (0.092) and days to first harvesting (0.049). Maximum significant negative correlation with yield per plant was revealed with number of primary branches (-0.253) and number of flowers per cluster (-0.258), number of fruits per plant exhibited negative non significant correlation with fruit yield per plant at phenotypic level. In case of genotypic level, the values of all twelve

characters were higher than phenotypic level. The results were in close harmony with Konyak *et al.* (2020) and Saha *et al.* (2019). Highly significant positive correlation was exhibited by fruit weight (0.856) and fruit girth (0.438) while fruit length (0.376), leaf length (0.358) and days to first flowering (0.283) exhibited significant positive correlation for trait yield at genotypic level and non significant but positive correlation was associated with fruit yield for days to first harvesting (0.089), plant height (0.092) and leaf width (0.091) at genotypic level.

Genotypic correlation was higher than phenotypic correlation coefficient and indicated that these attributes were mainly influencing the yield. Similar results regarding most of the traits reported here are in conformity with Sujin *et al.* (2017); Kumar *et al.* (2020); Bajpai *et al.* (2020).

5.1.6. Path coefficient analysis

Path coefficient analysis is employed to measure the direct and indirect effect of variables on yield. Yield is a complex trait and is dependent not only by the inter relationship of associated traits but also changes in any trait, that could affect the whole cause and affect the relationship of the characters. Path coefficient analysis has been carried out to analyse the direct and indirect effect of causal factors which affect the yield. It is simply standardized partial regression coefficient which splits the correlation coefficient into the measures of the direct and indirect effects of a set of independent variables on the dependent variable. In the present study, fruit weight (0.858), number of fruits per plant (0.443), fruit length (0.401), plant height (0.052), leaf length (0.080) and days to first flowering (0.018) imposed direct positive influence on fruit yield per plant at phenotypic level. Number of primary branches (-0.157), number of flowers per inflorescence (-0.094), leaf width (-0.045) and days to first harvesting (-0.066) imposed direct negative impact of fruit yield per plant at phenotypic level. Maximum positive direct effect on fruit yield per plant was exhibited by fruit weight (0.925), number of fruits per plant (0.460), leaf length (0.402), fruit length (0.275), plant height (0.700), days to first flowering (0.039) and days to first harvesting (0.021) at genotypic level which express maximum contribution in favour of increased fruit yield per plant. Leaf width (-0.228), number of primary branches (-0.157), fruit girth (-0.098) and number of flowers per inflorescence (-0.047) imposed negative direct effect on fruit yield at genotypic level. The

above findings are also preponderance with the conformity of Sujin *et al.* 2017; Konyak 2020; Saha *et al.* 2019 and Kumar *et al.* 2020.

The maximum and indirect positive effect on fruit yield per plant was participated by fruit weight (0.018), fruit girth (0.423), fruit length (0.365), and days to first flowering (0.210), days to first harvesting (0.049), plant height (0.092), leaf width (0.085) at phenotypic level whereas other characters showed negative indirect impact on fruit yield. Fruit weight imposed high positive indirect effect through days to first flowering (0.182), leaf length (0.241), leaf width (0.133), fruit girth (0.622) and days to first harvesting (0.140), whereas number of fruits per plant imposed maximum indirect positive effect via plant height (0.170) and number of primary branches (0.305). Fruit length showed higher positive indirect effect through leaf length (0.121), number of flowers per inflorescence, leaf width, number of primary branches, days to first harvesting and fruit weight imposed indirect positive effect on fruit yield. Leaf length imposed high positive indirect effect on leaf width (0.328), fruit length (0.140), fruit girth (0.128) and fruit weight (0.130) at genotypic level. These results are in line with the findings of Sujin *et al.* (2017); Kumar *et al.* (2020); Konyak *et al.* (2020); Saha *et al.* (2019) while other characters showed indirect negative impact on fruit yield.

5.1.7. Genetic divergence

5.1.7.1. Mahalanobis generalized distance

The concept D^2 introduced by Mahalanobis (1936) is neither restricted to population nor to the previously known population and the pattern obtained by D^2 analysis substantially change with addition of more characters. The technique is based on self weighing on genetic variability and gives clear idea about the diverse nature of the population. The cluster formed according to Tocher's method (Rao, 1952) was used to know the distance between and within the cluster. These results could give an insight diverse nature of the germplasm in a cluster. Cluster mean also give the degree of differences between germplasm which belong to different clusters. Mahalanobis D^2 statistics of multivariate analysis is recognized as a powerful tool in quantifying the degree of genetic divergence among the populations. Diverse the parents within a reasonable range, better are the chances of improving economic characters under consideration in the offspring.

Twenty two genotypes including two checks were included in the present study and it revealed that they have significant variation for all the traits taken under evaluation. The finding of the present study indicated a positive contribution of genetic divergence and yield components and this can be considered for selection of yield and yield attributing economic traits. The cluster contributing maximum to the divergence, one was given greater emphasis for deciding on the cluster for the purpose of further selection and the choice of the parents for hybridization. Based on D^2 statistics, all the twenty two genotypes grouped into five non overlapping clusters out of which cluster 1 have 17 genotypes which are maximum, while cluster 3 had two genotypes. Cluster 2, cluster 4 and cluster 5 had only one genotype each which indicates considerable diversity among the genotypes evaluated under this study. The genotypes sharing the same origin were grouped into different clusters and the genotypes from different origin were grouped in same cluster. The clustering pattern of the genotypes suggested no parallelism between genetic diversity and the geographical distribution of the genotypes. Clustering pattern of this finding is supported by Sadarunnisa *et al.* (2015); Yadav *et al.* (2017); Bhatt *et al.* (2019).

The intra and inter cluster distance were observed which represented index of genetic diversity among the traits. The greater intra cluster distance showed that there is maximum considerable amount of genetic diversity among the genotypes. The maximum intra cluster distance was observed in cluster 1 carrying 17 genotypes showed maximum intra cluster diversity ($D^2=159.36$) while, cluster 3 had two genotypes showing diversity with minimum value ($D^2=65.01$). Cluster 2, 4, and 5 had no intra cluster distance ($D^2=0.00$) as they possessed single genotypes which genetically varied from each other. In relation to inter cluster distance, cluster 3 recorded greater divergence with cluster 5 which indicated that the members of these two clusters are genetically very diverse to each other. The genotypes observed under this group could be applied for crop improvement through inter crossing. Minimum inter cluster distance was observed between cluster 1 and cluster 2 (385.17) which indicates close relationship between genotypes.

Higher degree of divergence among the genotypes within a cluster could produce more segregating breeding materials after hybridization, even selection within such cluster can be done based on maximum mean value for the desirable traits. Inter cluster distance of the genotypes were observed based on distance between the cluster and maximum inter cluster distance was observed between cluster 3 and cluster 4

($D^2=1538.29$) followed by cluster 2 and cluster 5 ($D^2=1057.25$), cluster 2 and cluster 4 ($D^2 = 791.34$), cluster 2 and cluster 3 ($D^2=756.87$) and cluster 2 and cluster 4 ($D^2=720.71$) indicated, the members of these two clusters are genetically very diverse to each other. The minimum inter cluster distance was recorded between cluster 1 and 2 ($D^2=385.17$) indicated that the genotypes of the clusters were not much genetically diverse from each other. Hence these genotypes can be used as single genotype in multiple crossing programmes for development of hybrid and segregating population. Sadarunnisa *et al.* (2015); Bhatt *et al.* (2019); Yadav *et al.* (2017); Bhusan *et al.* (2018); Karim *et al.* (2016); Dash *et al.* (2020) worked on the same aspect in various genotypes of brinjal and observed similar results for D^2 statistics for leaf length, number of fruits per plant, fruit length, fruit width, plant height, and fruit yield per plant and other related traits.

5.1.7.2 Cluster mean performance

The cluster means exhibited average cluster mean performance for most of the characters in general. In perusal of the data, cluster means of fruit yield per plant (2.53 kg) was highest because it had higher values for fruit weight, fruit girth, leaf width and leaf length, whereas lowest fruit yield per plant (0.05 kg) was observed by cluster 5 because the corresponding traits performed low, recorded lower values for the characters such as fruit girth, fruit length, leaf width, leaf length, days to 1st harvesting and also, the plant height found highest (197.33 cm) as compared to other genotypes and this contributed a negative role for fruit yield. Fruit yield per plant had average cluster means for the cluster 1, cluster 3 and cluster 4. Similar results were reported by Dash *et al.* (2020); Bhatt *et al.* (2019); Yadav *et al.* (2017); Bhusan *et al.* (2018); Karim *et al.* (2016); Sadarunnisa *et al.* (2015) in brinjal genotypes.

5.2 Screening of brinjal genotypes against bacterial wilt in field condition

Bacterial wilt caused by *Ralstonia solanacearum* in brinjal is the most devastating problem for successful cultivation in foothill areas. Twenty genotypes of brinjal along with two checks collected from North Eastern Region of India were screened to find out the resistant genotypes against bacterial wilt disease. Use of resistant varieties or genotypes which are resistant to this disease are some alternatives for successful

cultivation of brinjal in infected areas. The genotypes having good yield and yield related characteristics were screened against bacterial wilt.

5.2.1 Days to 1st incidence of bacterial wilt symptoms after inoculation

Days to first incidence of bacteria wilt was observed 6 days after inoculation in the genotype CHFB- 36, followed by 7 days after inoculation in CHFB- 27, CHFB-32 and CHFB- 37 while 15 days after inoculation in CHFB- 33 that showed late symptoms. This statement is supported by Namisy *et al.* (2019) where wilting symptoms first observed seven days after inoculation in most of susceptible eggplant accessions. Santhosha *et al.* (2015) observed that susceptible genotypes had susceptible reaction within 10 to 20 days after inoculation in the early growth stage of brinjal genotypes. Also, Hoque *et al.* (1981) reported high incidence of bacterial wilt 15 days after inoculation in tomato at the initial crop growth stage. Gopalakrishnan *et al.* (2014) recorded that the resistant brinjal accession (RES-5 and RES6) showed initial symptom after 14 days of the first inoculation.

5.2.2 Disease severity index (%)

In case of disease severity index, the minimum disease severity was recorded in CHFB-22, CHFB-29 and CHFB-33 (20%) indicated highly resistant against bacterial wilt. Severity index of this disease was maximum in CHFB-36 (84%) followed by CHFB-32 (76%), CHFB-27 (68%) and CHFB-37 (68%) showed susceptible. Disease severity index showed nil in the genotypes Arka Nidhi and Arka Keshav.

Based on rating scale against bacterial wilt of brinjal (0-5) by Winstead and Kelman (1952), 3 genotypes i.e., CHFB-22, CHFB-29, CHFB-33 grouped in one category showed resistant among these twenty two genotypes, 9 genotypes such as CHFB-21, CHFB-23, CHFB-24, CHFB-26, CHFB-28, CHFB-30, CHFB-31, CHFB-34 and CHFB-35 grouped in moderately resistant, 4 genotypes CHFB-20, CHFB-25, CHFB-18, CHFB-19 grouped in moderately susceptible, 3 genotypes CHFB-27, CHFB-32 and CHFB-37 grouped in susceptible and 1 genotype CHFB-36 recorded highly susceptible at field level.

5.2.3 Plant disease incidence (%)

Plant Disease Incidence was recorded at 1-15, 16-25, 25-30, 30-50 and 50-60 days after inoculation. CHFB-36 (83.33%) recorded maximum disease incidence 60 days after inoculation followed by CHFB-27, CHFB-32 and CHFB-37 (72.22%) while,

CHFB-22, CHFB-29 (20.00) recorded minimum disease incidence followed by CHFB-21, CHFB-23, CHFB-26 and CHFB-34 (33.33%). Gopalakrishnan *et al.* (2014) observed that the resistant brinjal accession (RES-5 and RES6) had 3.33% to 10.0% incidence of bacterial wilt. No incidence was seen in Arka Nidhi and Arka Keshav. Gopalakrishnan *et al.* (2014) also reported that there was no bacterial wilt incidence in resistant varieties, i.e., Arka Keshav and Arka Nidhi.

5.2.4 Cluster analysis

On the basis of its morphological traits against bacterial wilt, all the genotypes were grouped in two major clusters (I and II). Major cluster I was further divided into two sub clusters (IA and IB) and Major cluster II was further divided into two sub clusters (IIA and IIB). Arka Nidhi, Arka Keshav were grouped in sub cluster IA and showed highly resistant. This is similar with the results obtained by Chaudhary and Sharma (1999) where the brinjal varieties, Neelkanth, Arka Nidhi, Arka Kesav and SM 6-6 found resistant to bacteria wilt disease in their study.

Sub cluster IB included genotypes CHFB-20, CHFB-25, CHFB-18, CHFB-19, CHFB-22, CHFB-29, CHFB-33, CHFB-26, CHFB-34, CHFB-21, CHFB-24, CHFB-28, CHFB-31, CHFB-35, CHFB-30 and CHFB-23 which ranged from resistant, moderately resistant and moderately susceptible. Mondal *et al.* (2013) observed that local eggplant germplasm 'Midnapur Local' and 'Bhangar' were tolerant to the disease that was screened in bacterial wilt sick soil. CHFB- 27, 32 and CHFB- 37 recorded susceptible under sub cluster IIA. Genotype CHFB-36 was observed highly susceptible and grouped under sub cluster IIB.

The lines CHFB-22, CHFB-29 and CHFB-33 observed resistant in field condition, can be used in breeding programme for development of improved lines through hybridization. Results of the present study were in agreement with those obtained by Hussain *et al.* (2005) and Gopalakrishnan *et al.* (2014).

5.3 Assessment of bacterial wilt resistant brinjal genotypes based on molecular analysis.

Bacterial wilt is one of the major destructive diseases in brinjal and it has been observed that most of the varieties which are available in the market have moderate or low resistance to bacterial wilt. Therefore, there is a need to screen out genotypes

which are resistant to bacterial wilt. Hence, emphasis is laid on developing eggplant (brinjal) cultivars with resistance to *Ralstonia solanacearum*. Resistant varieties developed through breeding work is a time consuming process, therefore, molecular markers play an important role in the development of resistant varieties through Marker Assisted Selection (MAS). Simple sequence repeats (SSRs) are ideal DNA markers and generally more informative than other molecular markers such as RFLP due to its hyper-variability, co-dominant nature, multiallelic, reproducibility, genomic abundance, which can be used in this process (Rongwen *et al.*, 1995, Kalia *et al.*, 2010).

5.3.1 Molecular characterization using SSR markers

A total of 20 SSR primer pairs were used to identify and characterize the brinjal genotypes. The results revealed that the banding patterns produced were consistent and reproducible.

5.3.1.1 Generation of SSR profiles for identification

Genetic diversity of genotypes can be estimated by using molecular markers. SSR markers were found to be useful and vigorous tool for observing genetic diversity. These are PCR based, highly polymorphic, multi-allelic, co-dominant, easily reproducible and widely distributed across the genome (Powell *et al.* 1996). SSR profiles were generated using identified polymorphic SSR primer pairs to distinguish genotypes and to assess the genetic relatedness among the selected China aster genotypes. The selected primer pairs generated 40 alleles with an average of 3.07 alleles per locus at 50 to 320 bp range.

The present investigation demonstrated relatively high level of polymorphism *i.e.* out of 40 alleles, 35 were polymorphic showing 87.82 per cent of polymorphism. The possibility for high level of polymorphism in brinjal genotypes is due to their diverse nature. Similar results were obtained by Biswas *et al.* (2009) and Aguoru *et al.* (2015) in which 57.89% and 83.33% polymorphism in brinjal germplasm. Sharmin *et al.* (2011) and Islam *et al.* (2014) also reported 57.58% and 70.59% polymorphism in brinjal. Sultana *et al.* (2018) reported that the overall level of polymorphism (63.64%) indicated the effectiveness of markers to investigate genetic diversity among the different genotypes of brinjal.

In the present study, the number of alleles amplified using 20 polymorphic SSR primers ranged from 2 to 6 with an average of 3.007 which is similar to the result that recorded 2 to 11 alleles per locus for 30 eggplant accessions by using 19 SSR markers by Vilanova *et al.* (2014). Number of alleles ranged from 2 to 20 with an average of 4.67 was recorded by Verma *et al.* (2012) in brinjal. A total of 44 alleles were detected among the 48 brinjal genotypes with an average of 11 alleles per locus using SSR markers, which ranged from 9 to 14 by Ahmed *et al.* (2019). Caguiat and Hautea (2014) also reported 2 to 8 number of alleles with a mean of 4.3 alleles per marker in eggplant genotypes. Cericola *et al.* (2013) detected an average 7 alleles/locus in a study of 238 eggplants using four SSR markers.

5.3.2 Genetic diversity parameters of SSR primers

The different genetic diversity parameters such as polymorphic information content (PIC), marker index (MI), diversity index (DI), resolution power (RP) and percent polymorphism (100%) were analyzed. The ideal index to measure polymorphism is an average PIC value. A PIC value greater than 0.5 indicate loci of high polymorphism, value between 0.25 and 0.5 indicate loci of intermediate polymorphism and less than 0.25 indicate loci of low polymorphism (Ge *et al.*, 2013). Most of the primers were polymorphic and the polymorphic information content ranged from 0.25 to 0.75 with an average of 0.54 which indicate that the loci display intermediate to high polymorphism and occurrence of a wide gene pool among the genotypes. Similar results were obtained by Ahmed *et al.* (2019) in which PIC value ranged from 0.6413 to 0.8536 with an average of 0.7660. Vilanova *et al.* (2014) also recorded the polymorphic information content of SSR markers which is ranged from 0.07 to 0.77 with a mean value of 0.50. SSR markers were selected based on their PIC and high quality of bands as stated by Caguiat and Hautea (2014). Verma *et.al* (2019) recorded PIC value ranging from 0.32 to 0.75 with a mean of 0.54 in brinjal crop.

The primer emb01J19 (42.28) showed highest marker index (MI) value followed by primer SSR 2 (30.190) and primer emb01D10 (24.2) while, primer emb01E03 (5.1) showed lowest marker index (MI). The average marker index (MI) value observed was 17.73. The primer emb01J19 (0.95) showed highest diversity index (DI) value followed by primer SSR 2 (0.93) and primer emb01D10 (0.90). While, primer emb01E03 (0.62) showed lowest diversity index (DI) value. The average diversity index (DI) value was

0.82. The values obtained are in agreement with the findings of Ahmed *et al.* (2019) where they recorded the highest gene diversity value, 0.8672 and lowest value 0.6580 with a mean of 0.7847.

The maximum resolution power (RP) value observed was 2.00 for the primers emb01D10, primer emb01J19, eme25D01, emg11A06 and primer emf21E10 while, lowest value was observed for primer emb01E03 (0.60) followed by primer SSR 2 (1.45) with a mean of 1.73.

5.3.3. Assessment of genetic diversity

The study was undertaken to assess the genetic diversity and relatedness in brinjal genotypes using SSR markers.

A dendrogram was constructed based on cluster analysis of Nei M (1978) unbiased genetic similarity of SSR alleles among 22 genotypes and grouped into two clusters (I and II). Similar clustering were observed by Verma *et al.* (2019) where Unweighed Pair Group Method with Arithmetic Means (UPGMA) cluster tree analysis grouped 48 genotypes into seven major clusters by the use of SSR markers in brinjal crop. The genotypes that were derivatives of genetically similar type form cluster together.

The cluster I was further divided into two sub clusters, viz. IA and IB. Sub cluster IA was further divided into two sub-sub clusters IA₁ and IA₂. The sub-sub cluster IA₁ included the genotypes Arka Nidhi, Arka Keshav, CHFB-22, CHFB-26, CHFB-29, CHFB-21, CHFB-24, CHFB- 23, CHFB-31, CHFB-26, CHFB-28, CHFB-30 and sub-sub cluster IA₂ included CHFB-33. The genotypes Arka Nidhi and Arka Keshav of sub-sub cluster IA₁ are check varieties which are highly resistant against bacterial wilt. The genotypes Arka Kesav, Arka Neelkanth, Arka Nidhi and SM 6-6 were found resistant to bacterial wilt as reported by Chaudhary and Sharma (2000). The remaining genotypes of sub-sub cluster IA₁ i.e., CHFB-22, CHFB-26, CHFB-29, CHFB-21, CHFB-24, CHFB- 23, CHFB-31, CHFB-26, CHFB-28, CHFB-30 ranged from resistant to moderately susceptible grouped together with check varieties.

The genotype CHFB-33 which belongs to a different species i.e., *Solanum torvum* is observed to be resistant against bacterial wilt and is grouped under sub-sub cluster IA₂. *S. torvum* has been identified to carry traits of resistance to most serious diseases of eggplant, particularly bacterial and fungal wilts, and nematodes as reported by

Collonnier *et al.* (2001); Daunay *et al.* (1991); Stravato and Cappelli (2000); Clain *et al.* (2004).

Sub cluster IB included genotypes CHFB-34, CHFB-35, CHFB-19, CHFB-18, CHFB-37, CHFB-20, CHFB-25, CHFB-36. These genotypes ranged from moderately resistant to moderately susceptible except for CHFB-36. Although, the genotype CHFB-36 observed most susceptible in field condition, it was grouped under major cluster I in molecular analysis. This may be due to the similar genetic makeup of the genotype with other genotypes of major cluster I. Cluster II included the genotypes, CHFB-27 and CHFB-32 were found susceptible.

The inheritance of resistance to bacterial wilt in eggplant is still unclear as reported by Cao *et al.*, 2009 and these identified markers could be utilized for study as well as in the improvement of the lines by introgression of QTL for durable resistance.

Chapter-6

Summary and Conclusion

The present investigation entitled **“Screening of brinjal genotypes of North East India against Bacterial Wilt using SSR Markers”** was conducted with the following objectives:-

1. Screening of bacterial wilt severity in brinjal genotypes.
2. Assessment of bacterial wilt resistant brinjal genotypes based on molecular analysis.

This experiment was carried out at the polyhouse of College of Horticulture and Forestry, Central Agricultural University, Pasighat, Arunachal Pradesh during the year 2019-20. The experimental materials consisted of 22 genotypes of brinjal which were collected from different parts of north east region and maintained at farm of CHF, Pasighat. The data were collected based on 12 traits viz., days to 1st flowering, number of flowers per inflorescence, leaf length (cm), leaf width (cm), plant height (cm), number of primary branches, fruit length (cm), fruit girth (cm), days to first harvesting, number of fruit per plant, fruit weight (kg), fruit yield per plant (kg).

The data obtained were subjected to appropriate statistical analysis and results obtained are summarized below:

1. Analysis of variance revealed high and significant difference among the genotypes for all the characters under study.
2. The PCV was observed similar to GCV with higher values for most of the characters studied. The maximum PCV and GCV were found in fruit weight (70.90), number of fruits per plant (66.20), fruit girth (60.16) and fruit yield per plant (47.15). The minimum GCV and PCV were recorded for days to first flowering (8.06) and days to first harvesting (8.20). All other remaining characters showed medium GCV and PCV value.
3. Heritability was very high for all the characters studied viz. number of flowers per inflorescence, leaf width, plant height, number of primary branches, fruit length, fruit girth, number of fruits per plant, fruit yield per plant. Heritability was reported low in days to 1st harvesting, days to 1st flowering, and number of flowers per inflorescence.

Rest of the parameters showed medium heritability. Fruit weight and plant height showed highest genetic advance and lowest for fruit yield per plant (kg) and number of flowers per inflorescence.

4. High heritability coupled with high genetic advance was observed for fruit weight and fruit girth. These characters are therefore, governed by additive gene effects. It may also be concluded that selection on the basis of these characters will be more useful for the improvement of this crop towards attaining higher fruit yield per plant.
5. Studies revealed that fruit length (0.365) and leaf length (0.282) had significant and positive association with fruit yield per plant which indicates that importance of these characters in yield improvement through selection. Days to first flowering, leaf width (0.085), plant height (0.092) and days to first harvesting (0.049) was significantly and negatively correlated with fruit yield per plant which suggests that simultaneous improvement in population for fruit yield per plant from this character could not be achieved rather, independent selection should be practiced.
6. Path coefficient analysis revealed that fruit yield per plant had maximum positive direct effect with fruit weight followed by number of fruits per plant, fruit length, plant height, leaf length (0.080) and days to first flowering were the most important characters affecting fruit yield per plant both at phenotypic and genotypic levels. However, high negative direct effect was observed for leaf width, number of primary branches (-0.157), fruit girth (-0.098) and number of flower per inflorescence (at phenotypic and genotypic level). Hence, it would be rewarding to lay stress on these characters in selection programme for increasing the fruit yield per plant in brinjal.
7. In Mahalanobis D^2 analysis, among the five clusters, cluster 1 with seventeen genotypes formed the largest cluster followed by clusters 3 with two genotypes, cluster 2, 4 and 5 was unique, as it had only one genotype which indicates wider divergence among the genotypes. Intra cluster distance was highest in the cluster 1 (159.36) followed by cluster 3 (65.01) whereas the inter cluster D^2 value was maximum (1538.29) between cluster 3 and cluster 5. Hence, the material investigated showed high genetic variability and diversity among the genotypes.
8. Genetic diversity was assessed by using D^2 statistic. 22 genotypes were grouped into 5 clusters which had high range of inter cluster distance (D^2 value). Cluster 2 with genotype CHFB-18 were more divergent for days to 1st flowering, leaf width, fruit girth, fruit weight, fruit yield per plant. Cluster 5 with genotype CHFP-33 was found to

be promising lines for number of flowers per inflorescence, plant height and days to 1st harvesting. Cluster 4 with genotype CHFB-31 was identified as genetically divergent for number of primary branches and number of fruits per plant. Cluster 3 with genotypes CHFB-25, CHFB-26 was observed to be highly promising with parameters like leaf length and fruit length. Hence, the material investigated thus, showed high genetic variability and diversity among the genotypes. These superior genotypes could be used as parental sources in breeding programme for further improvement of the brinjal crop.

9. On the basis of morphological traits against bacterial wilt, 22 brinjal genotypes were grouped in two major as well as four sub clusters. Arka Nidhi, Arka Keshav were grouped in major cluster I, sub cluster IA and showed highly resistant. Genotype CHFB-36 grouped under major cluster II and sub cluster IIB was observed highly susceptible while CHFB-27, CHFB-32 and CHFB-37 were the susceptible lines recorded under major cluster II and sub cluster IIA. All other genotypes observed under major cluster I and sub cluster IB showed resistant, moderately resistant and moderately susceptible based on disease severity of bacterial wilt.
10. Early effect was seen by 6 days after inoculation of pathogen in the genotypes CHFB-36 followed by CHFB-27, CHFB-32 and CHFB-37 after 7 days while CHFB-33 showed late incidence after 15 days of inoculation. The disease severity was recorded minimum in CHFB-22, CHFB-29 and CHFB-33 (20%) indicated highly resistant against bacterial wilt. CHFB-36 (84%) followed by CHFB-32 (76%), CHFB-27(68%) and CHFB-37 (68%) showed maximum susceptibility. No incidence was seen in Arka Nidhi and Arka Keshav. Also, disease severity index showed nil in the genotypes Arka Nidhi and Arka Keshav.
11. On the basis of rating scale against bacterial wilt of brinjal (0-5) Winstead and Kelman (1952), genotypes CHFB-22, CHFB-29, CHFB-33 were grouped in one category which showed resistant among these twenty two genotypes while, CHFB-36 recorded highly susceptible. All other lines observed moderately resistant to moderately susceptible as measured on the rating scale. Hence, the genotypes CHFB-22, CHFB-29 and CHFB-33 observed resistant in field condition, can be used in breeding programme for development of resistant lines through hybridization.
12. A total of 20 SSR primers were used to analyze genetic diversity among 22 brinjal genotypes, and 40 alleles with allele size ranging from 50 to 320 bp were detected with an average of 3.07 alleles per locus. Out of 20 SSR loci, 13 were polymorphic

showing 87.82 per cent of polymorphism among 22 genotypes. Number of bands amplified by 13 primers ranged from 2 to 6 alleles.

13. Among genotypes, the polymorphic information content (PIC) value ranged from 0.25 to 0.75 with an average of 0.54 for different primer pairs. The maximum PIC value (0.75) was found in primer SSR 2 which was followed by emb01J19 primer (0.70). The least PIC value was found in primer emb01E03 (0.25), followed by primer SSR 1 (0.44). The maximum value for Marker Index (MI) was observed for the primer emb01J19 (42.28) followed by primer SSR 2 (30.19) and primer emb01D10 (24.20) while, lowest value for primer was emb01E03 (5.10) which is followed by primer emg11I04 (9.66), primer 11 emi04H10 (9.95). The maximum Diversity Index (DI) value was observed for the primer emb01J19 (0.95) followed by primer SSR 2 (0.93) and primer emb01D10 (0.90) while, lowest value was observed for primer emb01E03 (0.62) followed by primer emi04H10 (0.74) and primer emg11I04 (0.74). The maximum Resolution Power (RP) value was observed for the primer emb01D10 (2.00), primer emb01J19 (2.00), eme25D01 (2.00), emg11A06 (2.00) and primer emf21E10 (2.00) while, lowest value was observed for primer emb01E03 (0.60) followed by primer SSR 2 (1.45). Highest amount of percent polymorphism (100%) was reported by the primers EEMS46, SSR 1, SSR 2, emi04H10, emg11I04, emg11A06, emb01E03 and emf01D24 however, emf21E10 recorded the lowest (50%). Polymorphic Information Content, Diversity Index and Matrix Index were found to be highest in Primer emb01J19 and therefore, it can be concluded that Primer emb01J19 was the most efficient marker.
14. A dendrogram was constructed based on cluster analysis of Nei M (1978) unbiased genetic similarity of SSR alleles among 22 genotypes and grouped into two clusters (I and II). The cluster I was further divided into two sub clusters, IA and IB. Sub cluster IA was further divided into sub-sub clusters IA₁ and IA₂. The sub-sub cluster IA₁ included the genotypes Arka Nidhi, Arka Keshav, CHFB-22, CHFB-26, CHFB-29, CHFB-21, CHFB-24, CHFB- 23, CHFB-31, CHFB-26, CHFB-28, CHFB-30 and sub-sub cluster IA₂ included CHFB-33 and sub cluster IB included genotypes CHFB-34, CHFB-35, CHFB-19, CHFB-18, CHFB-37, CHFB-20, CHFB-25, CHFB-36. Cluster II included the genotypes, CHFB-27 and CHFB-32. The genotypes Arka Nidhi and Arka Keshav of sub-sub cluster IA₁ are highly resistant against bacterial wilt. The remaining genotypes of sub-sub cluster IA₁ i.e., CHFB-22, CHFB-26, CHFB-29, CHFB-21, CHFB-24, CHFB- 23, CHFB-31, CHFB-26, CHFB-28, CHFB-30 vary from

resistant to moderately susceptible. The genotype CHFB-33 belonging to a species *Solanum torvum* showed resistant and grouped under sub cluster IA₂. The genotypes of sub cluster IA i.e., CHFB-22, CHFB-26, CHFB-29, CHFB-21, CHFB-24, CHFB- 23, CHFB-31, CHFB-26, CHFB-28, CHFB-30, CHFB-34, CHFB-35, CHFB-19, CHFB-18, CHFB-37, CHFB-20, CHFB-25, CHFB-36 which are grouped together with check varieties vary from resistant to moderately susceptible. Although the genotype CHFB-36 observed most susceptible in field condition, it was grouped under major cluster I in molecular analysis. This may be due to the similar genetic makeup of the genotype with other genotypes of major cluster I. The genotypes under Cluster II, CHFB-27 and CHFB-32 were found susceptible.

15. The genotypes CHFB-22, CHFB-29, CHFB-33 were found resistant both in field screening and molecular analysis and therefore can be used in further breeding programme.

Suggestions for future work

1. Characters identified for high heritability coupled with high or moderate genetic gain in various genotypes may be considered for improvement through selection in brinjal.
2. Different traits such as fruit weight, number of fruits per plant, fruit length, plant height, leaf length and days to first flowering had direct effect with fruit yield per plant hence, these parameters need to be given importance for improvement in brinjal genotypes for further breeding programme.
3. Genotypes CHFB-18, CHFB-19 and CHFB-26 observed superior for fruit yield per plant may be tested in different locations to know their stability. If the above genotypes perform well, fruit yield could be recommended for general cultivation.
4. On the basis of field screening and SSR markers, the genotypes CHFB- 22, CHFB-29, CHFB-33 which found resistant against bacterial wilt can be utilized in the breeding program for further improvement of brinjal.
5. The SSR primers that produced polymorphic bands viz. EEMS46, SSR 1, SSR 2, emf21K08, emi04H10, emb01D10, emb01J19, eme25D01, emg11I04, emg11A06, emb01E03, emf21E10, emf01D24 can be suggested for further use in future experiments to screen against bacteria wilt.
6. Large number of primers needs to be screened to check for diversity of disease resistance in brinjal for better results.

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APPENDIX-I

Analysis of variance for different morphological characters in brinjal

S.N.	Source of variation	Mean square		
		Replication	Genotype	Error
	Df	2	21	42
1.	Days to 1 st flowering	0.31	45.87***	7.24
2.	Number of flowers per inflorescence	0.06	1.60***	0.06
3.	Leaf length (cm)	2.96	21.36***	2.4
4.	Leaf width (cm)	1.18	13.65***	1.37
5.	Plant height (cm)	306.86*	3328.45***	94.61
6.	No. of primary branches	0.56	4.61***	0.41
7.	Fruit length (cm)	0.02	47.63***	0.07
8.	Fruit girth (cm)	0.17	19.65***	0.16
9.	Days to first harvesting	2.60	70.91***	18.52
10.	Number of fruits per plant	0.04	113.26***	1.01
11.	Fruit weight (kg)	662.10	38262.90	297.12
12.	Fruit yield per plant (kg)	0.03	0.90	0.01

*** Significant at 1% probability level, * Significant at 5% probability level