

Original Research Article

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Choosing of Brinjal Parents for Future Breeding Aiming at Bacterial Wilt Disease Tolerance

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ABSTRACT

Brinjal production is threatened by bacterial wilt caused by *Ralstonia solanacearum* in the developing world. Evaluation in search of best performing genotypes against bacterial wilt disease and incorporating their genes in commercial cultivars is an effective attempt to the problem. Studies on genetic variability and heritability, along with degree of association between the various characters and direct effects of yield contributing characters on total yield, is of paramount significance in framing an appropriate breeding strategy. The proper choice of parent based on genetic divergence is a pre-requisite in plant breeding programme. In the present study reactions of 23 brinjal genotypes, in terms of bacterial wilt incidence differed at different days after transplanting (DAT). Thirteen genotypes showed resistant reactions, five genotypes were categorized as moderately resistant, and two genotypes each exhibited moderately susceptible and susceptible reaction up to 90 DAT. Resistant genotypes had comparatively lower mean fruit weight than other categorized groups, revealing the fact that small fruited genotypes are linked with disease resistance. High heritability coupled with high genetic advance was recorded in bacterial wilt incidence and other important traits indicating that the selection among the genotypes can bring about significant improvement in disease tolerance due to presence of additive genes in trait. The absence of relationship between genetic diversity and geographical distance indicated that selection of genotypes for hybridization should be based on genetic divergence rather than geographic diversity. Based on multivariate analysis and average values three elite brinjal genotypes 16/BRBW RES-2, 2012/BRBW RES-5 and 2012/BRBW RES-6, which had recorded high bacterial wilt resistance coupled with diverse horticultural traits, could be utilized as donor parents either for developing hybrids or to isolate promising lines, in segregating generation, resistant to this disease.

Keywords

Brinjal, Diversity,
Variability, PCA,
*Ralstonia
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Introduction

The pride of farmers, native to India, exhibiting wide diversity is the brinjal (*Solanum melongena* L.) possessing high production potential and belongs to every household kitchen throughout the year. India is one of the largest brinjal producing countries in the world covering an area of 0.66 million hectares with a production of 12.51 million tones and a productivity of 18.96 t/ha. West Bengal is the forerunner in brinjal production (30.03 lakh tonnes) with productivity of 18.53 t/ha (Anonymous, 2017).

However, successful cultivation is threatened by the attack of several insect pests, devastating diseases and growing of low yielding local types. Among plant diseases, soil-borne diseases are considered to be more limiting than seed-borne or air-borne diseases in the production of many crops and account for 10–20 % of yield losses annually (USDA, 2003). Bacterial wilt, a soil-borne disease caused by *Ralstonia solanacearum* (Yabucchi *et al.*, 1995) is a major limiting factor throughout tropical, sub-tropical and temperate regions of the world (Hayward, 1991). 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). In India, this disease is a serious threat in parts of Kerala, Orissa, Karnataka, Maharashtra, Madhya Pradesh and West Bengal (Rao *et al.*, 1976). Among solanaceous vegetables brinjal is more susceptible with yield losses ranging from 65 to 70% (Das and Chattopadhyay, 1953). Sudden wilting of the plant at flowering stage followed by yellowing of foliage and stunted plant growth are the characteristic symptoms (Kelman, 1953; Rai *et al.*, 1975) associated with brownish discoloration and rotting of vascular tissues (Smith, 1920). Initially this disease is

regarded as *R. solanacearum* species complex (RSSC) due to inclusion of a large number of genetic groups (Gillings and Fahy, 1994). Then RSSC is subdivided into five phylotypes viz., phylotype I strains originate from Asia, phylotype IIA strains from the north of Latin America and the Caribbean, phylotype IIB strains from South America, phylotype III strains from Africa, and phylotype IV strains from Indonesia, Australia, and Japan (Fegan and Prior, 2005). We need to fine tune our understanding with regard to molecular and genetic bases of resistance controlling the diversity of RSSC strains in order to breed brinjal for resistance to bacterial wilt. Three resistance QTLs (*EBWR2*, *EBWR14*, and *EBWR9*) control strains belonging to one or more phylotypes. By combining three resistance QTLs, breeders could able to develop varieties with a large spectrum of bacterial wilt resistance (Salgon *et al.*, 2017). Though resistance to bacterial wilt has been studied in several crops, especially tomato, scanty published works on bacterial wilt resistance in eggplant genotypes are available (Chaudhary and Sharma, 2000; Hussain *et al.*, 2005; Mondal *et al.*, 2013; Gopalakrishnan *et al.*, 2014).

Managing disease using appropriate farming practices needs further development and adaptation (Grimault and Prior, 1990). Hence, growing of resistant varieties could be an effective approach to the problem. Growing resistant brinjal is a durable, eco-friendly and effective control of the disease. Availability of and access to diverse genetic sources will ensure successful crop improvement programmes. Unfortunately, resistance reaction of commercial varieties is known to fluctuate both geographically and over time due to genetic variability of strains of the pathogens as well as the difference in the environments. So, it is necessary to evaluate different accessions in local conditions against the *R. solanacearum*. Therefore

extensive collection, characterization, evaluation for superior genotypes resistant to biotic/abiotic stresses and conservation of species diversity assumes great priority in crop improvement programmes of brinjal. Lack of resistance source of this disease in brinjal has forced breeders to look into the wild species (*Solanum torvum*) which have stable and reliable sources of resistance to bacterial wilt. However, the transfer of resistance from wild relatives is difficult by conventional breeding method to produce subsequent generations having good quality fruits. Hence, continuous search for new sources of resistance for the development of varieties/hybrids with higher level of resistance against bacterial wilt disease should be the prime objective.

Genetic improvement of any crop mainly depends on the amount of genetic variability present in the population and the germplasm serves as a valuable source of base population and provide scope for wide variability (Gavade and Ghadage, 2015). A good scope of improvement in economic traits relies on the extent of natural variation present in various characters among the genotypes. Variability is a combined measure of genetic and environmental causes. Variability parameters like genotypic and phenotypic coefficient of variations, heritability and genetic advance, along with degree of association between the various characters and direct effect of yield contributing characters on total yield, is of paramount significance in framing an appropriate breeding strategy that focus on exploiting the inherent variability that remains unaltered by environmental conditions among genotypes, which is more useful for exploitation in selection and hybridization. The proper choice of parent based on genetic divergence is a pre-requisite in plant breeding programme. In eastern part of India, very less number of bacterial wilt tolerant hybrids

developed so far in brinjal *vis-à-vis* the developed hybrids are not so popular with regard to fruit quality among the growers. Evaluation in search of best performing genotypes against bacterial wilt disease and incorporating their genes in commercial cultivars is an effective attempt to the problem.

Keeping in view the present study was undertaken to determine the extent of variability existing in the genotypes, to identify important selection indices and to select diverse genotype(s) that can be utilized in resistant breeding to retrieve potential high yielding lines resistant to bacterial wilt disease.

Materials and Methods

Plant material and field growing

Twenty-three genotypes of brinjal, collected from different sources were tested and evaluated under All India Coordinated Research Project on Vegetable Crops, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, West Bengal, India situated at 23.5° N latitude and 89° E longitude with a mean sea level of 9.75 m. Seed beds were prepared in a sandy loam soil and were 20 cm high and 1.0 m wide. Weathered cowdung manure at 4 kg/m² was mixed into the beds. Beds were drenched with a 4% formaldehyde solution and covered with a clear polythene sheet for 10 days to avoid damping off disease. Seed, after treatment with Thiram (3 g kg⁻¹ of seed), were sown during the 3rd week of July, 2017 at a depth 1 cm, were 5 cm apart, and covered with finely sieved well-rotted leaf mold (leaves left to decompose for two year) to add organic matter and to prevent the soil from drying. After sowing, beds were covered with straw until germination which normally takes five to seven days and hand watered everyday up to 2nd week of August. Nursery beds were

covered with 200 µm ultraviolet (UV)-stabilized clear polyethylene film supported by bamboo poles with open sides to protect seedlings from rain and direct sunlight. Seedlings were hardened by withholding water 4 days before transplanting. Thirty-day old seedlings were transplanted in sick field pre-inoculated with *R. solanacearum* during 3rd week of August following Randomized Block Design with three replications.

The population of *R. solanacearum* in the sick bed soil was estimated 8.46×10^8 cfu/g soil and soil pH was 6.5. The size of the experimental plot was 4.5 m × 4.2 m for each replication. The plant to plant and row to row distances were 75 cm and 60 cm, respectively accommodating 42 plants per plot. A fertilizer dose of 150 kg N (urea), 75 kg P (single super phosphate) and 75 kg K (muriate of potash) along with 10 tons of FYM/ha were incorporated in the soil as per schedule (Chattopadhyay *et al.*, 2011).

The bacterium, *R. solanacearum*, was isolated from diseased brinjal plants showing typical bacterial wilt symptoms on Triphenyltetrazolium chloride (TZC) medium. The seedlings of 23 brinjal genotypes were injured with sterilized knife and immersed in bacterial suspension containing *Ralstonia solanacearum* population of 1×10^8 cfu/g prior to transplanting into the main sick field. After inoculation, the plants were watered at alternate days and symptom of bacterial wilt was observed.

Data recording

The wilt symptoms and wilted plants were recorded at 30 days after transplanting (DAT), 60 DAT and 90 DAT and graded on a 0-5 scale of Winstead and Kelman (1952) with some modifications. The genotypes were classified into five different groups (Table 1)

according to percentage of wilted plants (PWP) as reported by Hussain *et al.*, (2005).

Observations were also taken from ten randomly selected plants with uniform fruits from each plot for qualitative characters fruit colour, calyx colour, fruit shape, and quantitative characters fruit length (cm), fruit diameter (cm), fruit weight (g), number of fruit per plant and fruit yield per plant (kg). The average values for each genotype in each replication for the traits studied were used for further statistical analysis.

Statistical analyses

Analysis of variance for the studied traits was analysed as per Panse and Sukhatme (1967). Genotypic and phenotypic coefficients of variation were calculated using the formulae of Burton (1952). Broad sense heritability was calculated as per Lush (1940) and genetic advance was estimated by the method of Johnson *et al.*, (1955). Categorization of genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV) and genetic advance (GA) were done as per Sivasubramanian and Menon (1973) and heritability by Johnson *et al.*, (1955). Phenotypic and genotypic correlation coefficients were computed as per Johnson *et al.*, (1955). Path analysis was carried out as per Dewey and Lu (1959). D^2 statistic was used for assessing the genetic divergence between populations (Mahalanobis, 1936). The grouping of the populations was done by using Tocher's method as described by Rao (1952). Hierarchical cluster analysis was performed with the same genotypes to observe degree of association according to characteristics expressed in a dendrogram (Ward, 1963). Principal component analysis (PCA), to identify the factor dimension of the data, was used to summarize varietal information in a reduced number of factors for selection of the best performing

genotype(s). Statistical analyses were done using SAS 9.3 Professional Version and SPSS Professional Version 13.0.

Results and Discussion

Reaction of genotypes against bacterial wilt disease

Brinjal genotypes were screened against *Ralstonia solanacearum* Race-I, Biovar 3. Wilting of plants was observed and the number of wilted plants was counted at 30 DAT, 60 DAT and 90 DAT (Table 2). Disease reactions were recorded based on the percentage of wilted plants from total population of 42 plants using the scale. Most of the researchers (Hussain *et al.*, 2005; Rahman *et al.*, 2011) have relied upon screening techniques of brinjal genotypes against bacterial wilt disease on the basis of percent disease incidence at different DAT. Reactions of different genotypes, in terms of incidence values of bacterial wilt differed at different DAT of brinjal. Normally, under field conditions, wilt symptom appears at the time of flowering, which is approximately 30 to 40 days after transplanting. Typical wilting symptom was first observed at 30 DAT in genotypes 16/BRBW RES-4, 16/BRBW RES-5, 16/BRBW RES-6, 16/BRBW RES-8, 13/BRBW/RES-1, 13/BRBW/RES-2, 13/BRBW/RES-3, PPL, 2012/BRBW RES-2, 2012/BRBW RES-4, 2012/BRBW RES-6, Arka Nidhi and Arka Kusumakar in varying degrees which escalated with increase in days while there was no symptom in genotypes 16/BRBW RES-1, 16/BRBW RES-2, 16/BRBW RES-3, 16/BRBW RES-7, 13/BRBW/RES-4, SM-6-7, 2012/BRBW RES-1, 2012/BRBW RES-3, 2012/BRBW RES-5 and 2012/BRBW RES-7 at 30 DAT. The ooze test showed the presence of xylem associated bacteria in plants of infected genotypes at 30 DAT. The incidence values among genotypes were lower (less than 10.00

%) in 2012/BRBW RES-5, 2012/BRBW RES-1, 16/BRBW RES-3, 13/BRBW/RES-4, 2012/BRBW RES-2, 2012/BRBW RES-3, 2012/BRBW RES-7, SM-6-7, 13/BRBW/RES-3, 2012/BRBW RES-4, 16/BRBW RES-1 and 2012/BRBW RES-6 and no incidence was recorded in 16/BRBW RES-2 up to 60 DAT. Thirteen genotypes 16/BRBW RES-1, 16/BRBW RES-2, 16/BRBW RES-3, 16/BRBW RES-5, 16/BRBW RES-7, 13/BRBW/RES-2, 13/BRBW/RES-3, 13/BRBW/RES-4, SM-6-7, 2012/BRBW RES-1, 2012/BRBW RES-3, 2012/BRBW RES-5 and Arka Nidhi showed resistant reactions with less than 20 % plants wilting, five genotypes 16/BRBW RES-8, 13/BRBW/RES-1, 2012/BRBW RES-4, 2012/BRBW RES-6 and 2012/BRBW RES-7 were categorized as moderately resistant with 21 - 40% plants wilting, two genotypes 16/BRBW RES-6 and PPL exhibited moderately susceptible with 41 - 60% plants wilting, whereas 16/BRBW RES-4 and Arka Kusumakar showed susceptible with 61-80% plants wilting at 90 DAT. The incidence value was lowest in 2012/BRBW RES-5 (6.35 %) which was statistically different from 16/BRBW RES-2 (8.33 %) and highest in Arka Kusumakar (77.78%) up to 90 DAT (Table 2).

The resistance exhibited by various genotypes may be due to the secondary metabolism of polyphenols, and the higher concentration of steroidal glycoalkaloids present in resistant plants, thereby preventing bacterial movement into the vicinity of the plant system as observed by Vasse *et al.*, (2005). Further, Prior *et al.*, (1994) reported that inhibitor extracts tyloses and gums in resistant plants act like filters, thereby preventing bacterial movement within a plant system. In previous studies, Hazra and Som (1999); Geetha and Peter (1993) and Ponnuswami (1999) also found varieties SM-6-7 and Arka Nidhi to be resistant in different environments. Host

resistance responses can be affected by environmental factors and the race and biovar. *Ralstonia solanacearum* causes lethal wilting disease in more than 200 plant species (Denny, 2000), while more than 450 plant species were listed as host plants for *R. solanacearum* including many important and economic crops (Hayward, 1991). The reason for these highly different and heterogeneous responses to the bacterial pathogen is not obvious; nevertheless it is assumed that specific pathogenic strains for certain hosts may have evolved only in certain parts of the world and are not found elsewhere or these hosts may only be susceptible where a number of environmental factors such as temperature, rainfall, soil type, inoculums and other soil biological factors are conducive to disease expression coincide (Hayward, 1991). Hanson *et al.*, (1996) while studying the field reaction of tomato lines to different strains of *R. solanacearum* found that in Malaysia and Taiwan most of the tomato lines were resistant, but in the Philippines and Indonesia they were susceptible. This indicates that it is necessary to evaluate different accessions in local conditions against the *R. solanacearum*.

Resistant genotypes that possess suitable horticultural traits are a breeder's concern in crop improvement programme especially in disease prone areas. Fruit yield of genotypes varied from 241.46 q/ha (Arka Nidhi) to 308.64 (16/BRBW RES-2) among resistant genotypes and 120.27 q/ha (2012/BRBW RES-2) to 250.37 q/ha (16/BRBW RES-8) among moderately resistant genotypes (Table 2). The highest fruit yield was recorded in 16/BRBW RES-2 (308.64 q/ha) followed by 16/BRBW RES-7 (296.42 q/ha) and 16/BRBW RES-3 (293.21 q/ha). Despite the genotype 2012/BRBW RES-5 exhibited the lowest disease incidence, but it ranked 8th in respect to yield potential. It can be concluded that genotype with low incidence of bacterial wilt disease does not always perform better

with respect to yielding ability. The lowest fruit yield was recorded in Arka Kusumakar (SC) with only 80.88 q/ha and had highest disease incidence of 77.78% followed by PPL (57.10%) with 114.42 q/ha yield.

The genotypes varied greatly in qualitative parameters such as fruit colour, calyx color and fruit shape (Table 3). Genotypes with high yield coupled with low disease incidence have purple colour fruits with green calyx which are most appealing characters from consumers' point of view particularly in eastern India. In view of changing local preference for colour, shape, taste, etc., it is not possible to have one common cultivar to suit different localities of a region. It is, therefore, necessary to improve the locally preferred cultivars or their utilization to develop new hybrid combinations for high yield, quality, disease tolerance, consumer acceptability and to meet diverse taste of local people.

Genotypes were highly significant for different fruit characters under study (Table 4). Fruit length of genotypes varied greatly (9.52 cm to 27.45 cm), the maximum being in 2012/BRBW RES-6 while, the minimum was observed in 16/BRBW RES-1. The length of fruit had no direct bearing among disease reaction groups. The maximum diameter of fruit was recorded in 16/BRBW RES-5 (7.50 cm) and minimum in 16/BRBW RES-6 (3.00 cm). The maximum variation in fruit weight (58.00 g to 225.67 g) among genotypes was observed. The highest fruit weight was recorded in 16/BRBW RES-5 followed by 16/BRBW RES-3 and the lowest fruit weight was observed in 13/BRBW/RES-3. Genotypes showing disease resistant reactions had comparatively lower mean fruit weight (105.71 g) ranging from 85.33 g to 126.67 g than other categorized groups. The maximum number of fruit per plant was produced by 13/BRBW/RES-3 (21.11) and the minimum

was recorded in 2012/BRBW RES-7 (3.63). Wide variations in quantitative characters of brinjal genotypes tested previously in the Gangetic plains of West Bengal were also recorded by Das *et al.*, (2010), Chattopadhyay *et al.*, (2011), Shende *et al.*, (2016) and Dutta *et al.*, (2018). It was found that bacterial wilt resistance in brinjal is tightly linked with small fruit size. Scott *et al.*, (2005) observed that bacterial wilt resistance originated from wild tomato, particularly *Lycopersicon esculentum* var. *cerasiforme* and *L. pimpinellifolium* and most tomato resistance sources tend to be small-fruited. Combining a complete set of desirable traits including horticultural characteristics and resistance to bacterial wilt into a single variety is rather challenging and sometimes can be contradictory (Guan *et al.*, 2012). The close linkage between resistance against bacterial wilt in the Solanaceae and small fruit size is such an example (Louws *et al.*, 2010).

Genetic variability and heritability

The genotypic coefficient of variation (GCV) helps to measure the range of genetic variability in a character and provides a measure to compare genetic variability present in various characters. Heritable variation cannot be measured using GCV alone. Estimates of GCV and PCV corresponded well for the expression of all characters under study indicating lesser influence of environment. PCV were slightly higher than the corresponding GCV indicating prevalence of environmental influence on expression of these traits (Table 5). However, the difference between PCV and GCV being very low for majority of the characters indicated the existence of inherent variability that remains unaltered by environmental conditions among genotypes, which is more useful for exploitation in selection and hybridization. High GCV and PCV values occurred for all characters indicating the

potential of simple selection for improvement of these characters. These results are in conformity with Banerjee *et al.*, (2018) and Parvati *et al.*, (2018). A high proportion of GCV to PCV is desirable in selection because it indicates the traits are under genetic, rather than environmental control (Kaushik *et al.*, 2007).

Heritability is of interest to plant breeders primarily as a measure of the value of selection for a particular character in various types of progeny, and as an index of transmissibility of characters from parent to offspring (Hayes *et al.*, 1955). The concept of heritability is important to evaluate relative magnitude of effects of genes and environments on total phenotypic variability. For this reason, Burton (1952) stated that genetic variability, along with heritability, should be considered to assess the maximum and accurate effect of selection. Broad sense heritability value was highest for BWI (99.69%) followed by fruit weight (99.11%), number of fruits/plant (93.99%), fruit yield/plant (90.26%), fruit length (88.74%) and fruit diameter (60.12%) (Table 5). These observations corroborate the findings of Patel *et al.*, (2004) for fruit length, fruit diameter, fruit weight and yield/ plant, Singh and Kumar (2005) for average fruit weight and fruit yield/ plant, Naliyadhara *et al.*, (2007) for fruit diameter and fruit length, Muniappan *et al.*, (2010) for fruit length, fruit breadth and average fruit weight, Rad *et al.*, (2015) for fruit weight and fruit length. High magnitude of heritability was also reported for fruit diameter, fruit length, fruit weight and fruits/plant by Kushwah and Bandhopadhyaya (2005), Ambade *et al.*, (2013) and Manpreet *et al.*, (2013) and Banerjee *et al.*, (2018). High heritability for fruit diameter, fruit weight, number of fruits/ plant and yield/ plant was also reported by Babu and Patil (2005), Karak *et al.*, (2012), Mili *et al.*, (2014) and Parvati *et al.*, (2018). High heritability indicates that the

environmental influence is minimal on characters and these characters can be used for selection.

Genetic advance is improvement in performance of selected lines over the original population. It is not necessarily true that high heritability would always exhibit high genetic advance. For this reason, Johnson *et al.*, (1955) stated that heritability in combination with genetic advance would be more reliable for predicting the effects of selection because genetic advance depends on the amount of genetic variability, magnitude of masking effect of genetic expression (environmental influence), and intensity of selection. In this present study, high magnitude (> 20.00%) of genetic advance as per cent of mean was recorded in characters BWI, fruit length, fruit diameter, fruit weight, number of fruits/plant, and fruit yield/plant (Table 5). Parvati *et al.*, (2018) and Banerjee *et al.*, (2018) also reported high genetic advance fruit length (cm), fruit diameter (cm), fruit weight (g), number of fruits/plant, BWI (%) and fruit yield/plant (kg). High heritability coupled with high genetic advance was recorded in BWI (%), fruit length (cm), fruit diameter (cm), fruit weight (g), number of fruits/plant and fruit yield/plant (kg) indicating that the selection among the genotypes can bring about significant improvement in the fruit yield and its component characters due to the presence of additive genes in traits. This result agreed with the findings of Ravali *et al.*, (2017), Banerjee *et al.*, (2018) and Parvati *et al.*, (2018). High heritability estimates with high GA was also reported by Tirkey *et al.*, (2018) and Prasad *et al.*, (2004) for fruit yield and fruit weight; Babu and Patil (2008), Mili *et al.*, (2014) and Sharma and Swaroop (2000) for fruit weight, fruits per plant and yield. Estimates of heritability with genetic advance are more reliable and meaningful than individual consideration of the parameters.

Character association

Most correlation coefficients at the genotypic level were greater than the corresponding phenotypic ones (Table 6). The higher values of genotypic, than phenotypic, correlation indicated that the genotypic effects were more important than environmental factors (Falconer 1988). In the presence of high environmental influence on expression of characters, there is the possibility of over estimation of the genotypic correlation coefficient. Only number of fruits/plant exhibited significantly positive genotypic correlation with fruit yield/plant. Positive correlation between fruit yield and number of fruits/plant was also recorded by earlier workers (Samlindsujin *et al.*, 2017; Dutta *et al.*, 2018; Banerjee *et al.*, 2018). Percentage of bacterial wilt incidence was negatively correlated with fruit yield/plant. This indicated that lower the incidence of bacterial wilt will result in higher fruit yield/plant.

Fruit weight and number of fruit per plant had positive, direct effects on fruit yield/plant (Table 6), likely due to positive association with fruit yield/plant. Similar trend of findings were also obtained with other genotypes and environmental conditions by Thangamani and Jansirani (2012), Muniappan *et al.*, (2010), Banerjee *et al.*, (2018), Samlindsujin *et al.*, (2017); and Rad *et al.*, (2015) for average fruit weight in eggplant. Percentage of bacterial wilt incidence (BWI %) had negative direct effects on fruit yield/plant due to negative association with fruit yield/plant. Direct effects of other characters were negligible. Direct selection could be beneficial for yield improvement since number of fruit/plant and fruit weight exhibited significant, positive, correlations with fruit yield/plant. The residual effect was low indicating inclusion of maximum important fruit yield/plant influencing characters in the analysis.

Table.1 Disease rating scale of bacterial wilt disease in brinjal

Scale	Reaction	Percentage of wilt
0	Highly resistant (HR)	Plants did not show any wilt symptom
1	Resistant (R)	1-20% plants wilted
2	Moderately resistant (MR)	21-40% plants wilted
3	Moderately susceptible (MS)	41-60% plants wilted
4	Susceptible (S)	61-80% plants wilted
5	Highly susceptible (HS)	More than 80% plants wilted

Table.2 Wilt reaction at periodic interval and fruit yield (q/ha) of brinjal genotypes

Genotypes	Bacterial wilt incidence (%)			Reaction at 90 DAT	Fruit yield (q/ha)
	30 DAT	60 DAT	90 DAT		
16/BRBW RES-1	0.00	8.33 (16.78)	13.89 (21.88)	R	278.09
16/BRBW RES-2	0.00	0.00	8.33 (16.78)	R	308.64
16/BRBW RES-3	0.00	2.79 (9.62)	11.11 (19.47)	R	293.21
16/BRBW RES-4	16.67 (24.10)	47.21(43.40)	63.89 (53.06)	S	192.53
16/BRBW RES-5	5.55 (13.63)	11.12 (19.48)	19.44 (26.16)	R	271.36
16/BRBW RES-6	14.6 (22.46)	38.88 (38.57)	58.33 (49.80)	MS	212.1
16/BRBW RES-7	0.00	5.55 (13.63)	16.67 (24.10)	R	296.42
16/BRBW RES-8	5.55 (13.63)	15.29 (23.02)	22.22 (28.12)	MR	250.37
13/BRBW/RES-1	4.76 (12.60)	20.95 (27.24)	35.10 (36.33)	MR	185.34
13/BRBW/RES-2	1.90 (7.92)	11.43 (19.76)	19.00 (25.84)	R	245.51
13/BRBW/RES-3	2.38 (8.87)	7.86 (16.28)	16.10 (23.66)	R	273.77
13/BRBW/RES-4	0.00	6.67 (14.97)	14.90 (22.71)	R	292.33
SM-6-7	0.00	7.14 (15.50)	17.30 (24.58)	R	246.24
PPL	11.43 (19.76)	28.1(32.01)	57.10 (49.08)	MS	114.42
2012/BRBW RES-1	0.00	1.60 (7.27)	15.08 (22.85)	R	225.34
2012/BRBW RES-2	1.6 (7.27)	6.36 (14.61)	23.81 (29.21)	MR	120.27
2012/BRBW RES-3	0.00	6.36 (14.61)	12.70 (20.88)	R	248.17
2012/BRBW RES-4	2.38 (8.87)	8.74 (17.20)	24.60 (29.73)	MR	165.46
2012/BRBW RES-5	0.00	1.60 (7.27)	6.35 (14.60)	R	251.51
2012/BRBW RES-6	2.38 (8.87)	9.52 (17.97)	25.40 (30.26)	MR	198.11
2012/BRBW RES-7	0.00	7.93 (16.36)	22.22 (28.12)	MR	152.98
Arka Nidhi (RC)	2.79 (9.62)	12.50 (20.70)	16.67 (24.10)	R	241.46
Arka Kusumakar (SC)	22.21(28.12)	61.12(51.43)	77.78 (61.88)	S	80.88
C.D. at 5%	0.842	0.983	1.127	-	25.32
C.V. (%)	6.32	2.99	2.29	-	14.27

*Figures in parentheses are angular transformed values.

R = Resistant; MR = Moderately resistant; MS = Moderately susceptible; S = Susceptible

RC= Resistant Check; SC= Standard Check

Table.3 Qualitative characters of 23 genotypes of brinjal genotypes

Entries	Fruit colour	Calyx colour	Fruit shape
16/BRBW RES-1	Purple	Green with purple mix	Oblong
16/BRBW RES-2	Purple	Green	Long
16/BRBW RES-3	Green with white stripes at basal end	Green	Oblong
16/BRBW RES-4	Green	Green	Long
16/BRBW RES-5	Green with purple stripes at blossom end	Green	Oblong
16/BRBW RES-6	Light green	Green	Long
16/BRBW RES-7	Purple	Green	Long
16/BRBW RES-8	Green	Green	Long
13/BRBW/RES-1	Green with white stripes at blossom end	Green	Long
13/BRBW/RES-2	Purple	Green	Long
13/BRBW/RES-3	Light green	Green	Long
13/BRBW/RES-4	Greenish purple	Green	Long
SM-6-7	Whitish green	Green	Long
Pusa Purple Long	Purple	Green	Long
2012/BRBW RES-1	Purple	Green	Long
2012/BRBW RES-2	Green	Green	Long
2012/BRBW RES-3	Green with white stripes at blossom end	Green	Long
2012/BRBW RES-4	Purple	Green	Long
2012/BRBW RES-5	Purple	Green	Long
2012/BRBW RES-6	Purple with greenish shade	Green	Long
2012/BRBW RES-7	Purple	Green	Long
Arka Nidhi	Blackish Purple	Green	Long
Arka Kusumakar	Green	Green	Long

Table.4 Fruit characters of brinjal genotypes

Entries	Fruit length (cm)	Fruit diameter (cm)	Fruit weight (g)	Number of fruits/plant
<i>Resistant group</i>				
16/BRBW RES-1	9.52	5.85	85.33	18.32
16/BRBW RES-2	18.46	3.80	92.67	20.49
16/BRBW RES-3	10.32	6.10	120.67	10.93
16/BRBW RES-5	12.54	7.50	125.52	9.71
16/BRBW RES-7	19.27	4.28	126.67	10.49
13/BRBW/RES-2	17.25	3.85	87.00	12.66
13/BRBW/RES-3	12.90	3.20	58.00	21.11
13/BRBW/RES-4	15.37	4.00	123.00	8.94
SM-6-7	18.20	4.25	115.00	9.98
12/BRBW RES-1	20.01	4.35	120.00	10.44
12/BRBW RES-3	16.80	5.10	120.00	8.41
12/BRBW RES-5	23.25	3.14	95.00	12.79
Arka Nidhi	14.37	4.62	105.33	10.84
Mean	16.02	4.62	105.71	12.70
<i>Moderately resistant group</i>				
16/BRBW RES-8	17.85	3.87	115.33	9.76
13/BRBW/RES-1	14.85	6.01	172.00	5.11
12/BRBW RES-2	16.54	4.31	155.00	3.35
12/BRBW RES-4	23.20	4.45	150.00	4.93
12/BRBW RES-6	27.45	4.31	210.00	4.15
12/BRBW RES-7	13.59	7.04	196.00	3.63
Mean	18.91	5.00	166.39	5.16
<i>Moderately susceptible group</i>				
16/BRBW RES-6	14.36	3.00	145.30	6.53
Pusa Purple Long	22.70	3.55	120.00	5.27
Mean	18.53	3.28	132.65	5.90
<i>Susceptible group</i>				
Arka Kusumakar	14.25	3.87	93.00	3.85
16/BRBW RES-4	14.65	3.57	162.33	5.35
Mean	14.45	3.72	127.67	4.60
C.D. at 5%	2.53	1.20	15.32	3.27
C.V. (%)	7.52	4.37	11.20	10.34

Table.5 Mean, range, and estimates of genetic parameters of brinjal genotypes

Characters	Mean	Range	GCV	PCV	GCV: PCV (%)	Heritability in b.s (%)	GA as % mean
Fruit length (cm)	16.99	9.52 - 30.45	27.30	28.98	94.20	88.74	52.98
Fruit diameter (cm)	4.52	3.0 - 6.10	24.90	32.12	77.54	60.12	39.78
Fruit weight (g)	129.71	58.0 - 225.67	38.86	39.04	99.55	99.11	79.70
Number of fruits/plant	9.79	3.85 - 21.11	50.40	51.98	96.95	93.99	100.65
Bacterial wilt incidence (%)	26.00	6.35 - 77.78	41.21	41.27	99.84	99.69	84.76
Fruit yield / plant (kg)	0.94	0.36 - 1.33	27.76	29.22	95.00	90.26	54.32

GCV= Genotypic Coefficient of Variance, PCV= Phenotypic Coefficient of Variance, GA= Genetic Advance

Table.6 Phenotypic and genotypic correlations among six characters and their direct effects on yield/plant

Characters	rg	rp	Direct effects on yield/plant
Fruit length (cm)	-0.215	-0.200	-0.140
Fruit diameter (cm)	0.057	0.099	-0.120
Fruit weight (g)	-0.084	0.082	0.569
Number of fruits/plant	0.552*	0.494*	0.738
Bacterial wilt incidence (%)	-0.741**	-0.701**	-0.433

*, ** significant at P<0.05 and P<0.01, respectively.

rg = Genotypic correlation coefficient; rp = Phenotypic correlation coefficient.

Residual are 0.26906

Table.7 Cluster classification and source of collection of brinjal genotypes

Number of clusters	Name of the genotype
I (2)*	Arka Kusumakar (SC), Pusa Purple Long
II (4)	16/BRBW RES-1,16/BRBW RES-2, 13/BRBW/RES-2, 2012/BRBW RES-5
III (5)	16/BRBW RES-7, 13/BRBW/RES-1, 13/BRBW/RES-4, 2012/BRBW RES-3, 2012/BRBW RES-4
IV (2)	16/BRBW RES-4, 16/BRBW RES-6
V (4)	16/BRBW RES-3, 16/BRBW RES-5, 2012/BRBW RES-6, 2012/BRBW RES-7
VI (5)	16/BRBW RES-8, Arka Nidhi (RC), SM-6-7, 2012/BRBW RES-1, 2012/BRBW RES-2
VII (1)	13/BRBW/RES-3

*Figures in parentheses indicate number of genotypes

Table.8 Inter-cluster distances of twenty five genotypes of brinjal

Cluster	II	III	IV	V	VI	VII
I	57.302	78.942	37.219	120.097	50.502	68.99
II		75.004	55.125	121.161	25.654	33.456
III			107.034	46.539	50.08	107.379
IV				151.405	64.743	46.213
V					96.55	153.58
VI						57.803

Table.9 Cluster means of six characters of brinjal genotypes

Character	I	II	III	IV	V	VI	VII
Fruit length (cm)	22.7	17.25	14.85	14.65	12.54	20.1	12.9
Fruit diameter (cm)	3.55	3.85	6.01	3.57	7.75	4.35	3.2
Fruit weight (g)	108	87	172	62.33	225.67	120	58
Number of fruit per plant	4.77	12.7	4.85	13.9	5.41	8.45	21.24
Bacterial wilt incidence (%)	57.1	19.0	35.1	63.89	19.44	15.08	16.10
Fruit yield per plant (kg)	0.52	1.1	0.83	0.87	1.22	1.01	1.23

Table.10 Results of principal component analysis (PCA) for four quantitative characters

Principal Component (PC)	Eigenvalue	% Variance	% Cumulative variance
Eigenvalues and variance accounted for (%) by PCA based on correlation matrix			
Fruit length (cm)	2602.53629	87.76	87.76
Fruit diameter (cm)	333.67528	11.25	99.01
Fruit weight (g)	23.51928	0.79	99.80
Bacterial wilt incidence (%)	5.51366	0.19	99.99

Table.11 Contribution of diverse traits in the principal components of brinjal

Variables	PC ₁	PC ₂	PC ₃	PC ₄
Factor loadings due to PCs with eigenvalues > 1				
Fruit length (cm)	0.010255	0.001888	0.964372	0.222814
Fruit diameter (cm)	0.018005	0.000408	-0.125907	0.086545
Fruit weight (g)	0.988528	0.127445	-0.025978	0.075273
Bacterial wilt incidence (%)	-0.135365	-0.985043	-0.025579	-0.103017

Fig.1 Dendrogram of genotypes of brinjal following Ward’s method. Genotypes are in left most columns

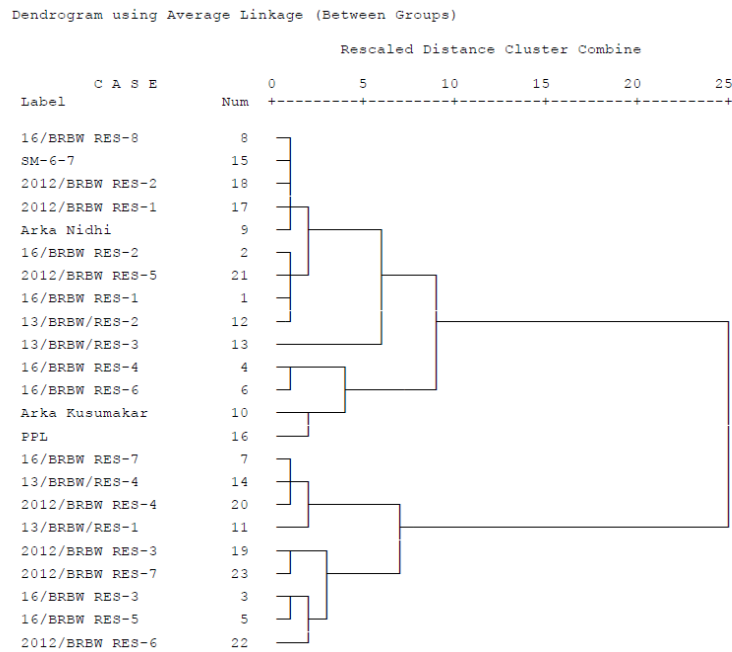
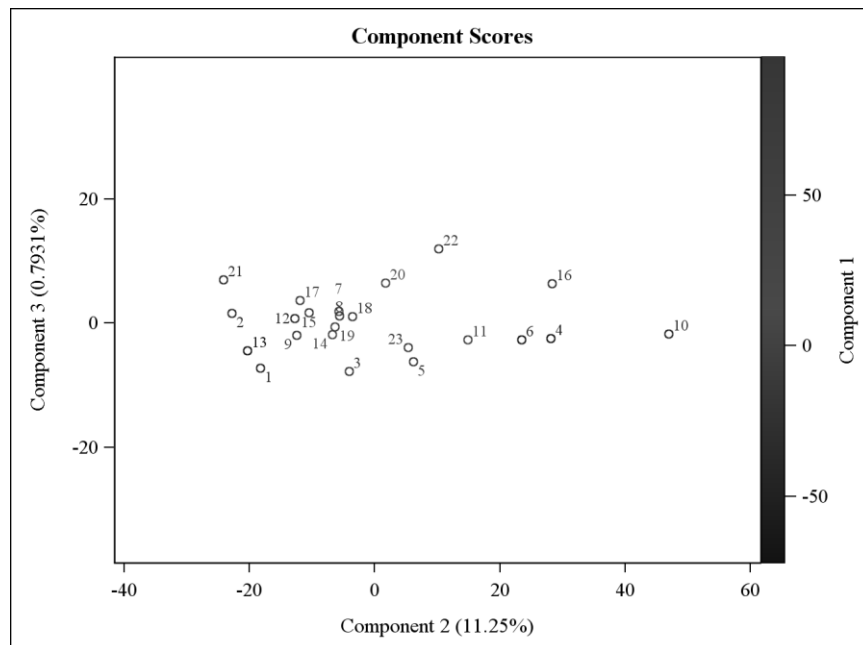


Fig.2 Scatter diagram of regression factor scores for the second and third components as determined by principal component analysis. Points in diagram closest to the intersection of 0 on the X- and Y-axes indicate similarity. Outliers on the X-axis, i.e., 1= 16/BRBW RES-1, 2 = 16/BRBW RES-1, 13 = 13/BRBW RES-3, 21 = 12/BRBW RES-5, 6 = 16/BRBW RES-6, 16 = PPL and 10 = Arka Kusumakar, indicate diversity. Numbers correspond to name of the genotype (see Figure 1)



Genetic diversity through multivariate analysis

Assessment of divergence for a set of characters utilizing different multivariate analyses has been effectively utilized in vegetable crops with diverse breeding system (Murthy, 1979). Kalloo *et al.*, (1980) suggested that the crosses between selected genotypes from widely separated clusters were most likely to give desirable recombinants. Based on the determination of divergence, all the twenty three genotypes could meaningfully be grouped into seven clusters (Table 7). Cluster III and VI had the maximum genotypes i.e. 5 genotypes in each clusters, Cluster II and VI comprised of 4 genotypes each, while Cluster I and Cluster IV had 2 genotypes each and cluster VII had 1 genotype.

The genetic divergence among brinjal genotypes through cluster analysis was reported by many workers (Begum *et al.*, 2013; Banerjee *et al.*, 2018; and Dutta *et al.*, 2018). In general, the pattern of distribution of genotypes from diverse geographical region into different clusters was random. It might be due to free and frequent exchange of genetic materials among the farmers and breeders of different regions. Differential selection pressure according to regional preference also produced greater uniformity in the germplasm. The absence of relationship between genetic diversity and geographical distance indicated that forces other than geographical origin such as exchange of genetic stock, genetic drift, spontaneous mutation, natural and artificial selection were responsible for genetic diversity. Therefore, the selection of genotypes for hybridization should be based on genetic divergence rather than geographic diversity. Environmental influence on the composition of cluster was also recorded earlier in different self-pollinated crops like brinjal (Madhavi *et al.*,

2015), tomato (Peter and Rai, 1976), pea (Kalloo *et al.*, 1980) and cowpea (Hazra *et al.*, 1992).

The inter-cluster distance represents the index of genetic diversity among clusters (Table 8). The inter-cluster distance among 23 genotypes revealed that the minimum value was observed between Cluster I and IV (57.008) indicating close relationship among the genotypes included in these clusters. The maximum inter cluster value was observed between cluster V and VII (153.58) followed by 151.405 between Cluster IV and V, which indicated that the genotypes included in these clusters had the maximum divergence. Hence, intermating between the genotypes included in these clusters will be expected to give transgressive segregates in the advanced generation. Kalloo *et al.*, (1980) suggested that the crosses between selected varieties from widely separated clusters were most likely to give desirable recombinants.

Cluster mean based estimations are very useful in targeting the genotypes for breeding programme, as they prevent the tedious efforts of screening the inferior germplasm lines. Hence, genotypes from desirable clusters could be directly used for final field evaluation in advanced breeding experiments. The character means were worked out for the genotypes falling in these seven clusters (Table 9) showed that the mean values of the clusters varied in magnitude for all the six characters. Cluster VII was the highest yielder followed by cluster V. Cluster VI had lowest bacterial wilt incidence while highest belongs to genotypes in cluster IV. Regarding fruit weight, cluster V showed highest performance followed by cluster III. Highest number of fruits per plant was produced in the genotypes belonging to cluster VII followed by the genotypes under cluster IV. These clusters could be regarded as useful sources of gene for important yield component traits.

Hence, it can be suggested from the present study that a high yielding brinjal type with appreciable bacterial wilt disease tolerance could be bred by utilizing the genotypes from cluster VII and VI as parents in future breeding.

The PCA was performed to obtain a simplified view of the relationship between the characters fruit length, fruit diameter, fruit weight, bacterial wilt incidence and number of fruits per plant as shown in Table 10. Variable loadings for components PC1 (Fruit length), PC2 (Fruit diameter), PC3 (Fruit weight) and PC4 (Bacterial wilt incidence) were chosen because their eigenvalues exceeded 1.0 and explained 99.99% of the total variance (Table 11).

The first component (PC1) explained 87.76% of total accounted for variance in which an increase in fruit length (cm) leads to increase in fruit diameter (cm), fruit weight (g), fruit and a decrease in percentage of bacterial wilt incidence (Table 11). The second component (PC2) explained an additional 11.25% of the variance in which an increase in fruit length (cm) was associated with increased fruit diameter, fruit weight along with decreased percentage of bacterial wilt incidence. The PCA was used to determine the strength of relationship between variables among brinjal genotypes by Ullah *et al.*, (2014), Rad *et al.*, (2015), Karim *et al.*, (2016), Banerjee *et al.*, (2018) and Dutta *et al.*, (2018). There are no clear guidelines to determine the importance of a trait coefficient for each principal component. Johnson and Wichern (1988) regard a coefficient greater than half of the coefficient, divided by the square root of the standard deviation of the eigenvalue of the respective principal component, as significant.

High diversity occurred among genotypes along with strong relationships (Figure 1).

The scatter diagrams (Figure 2) indicated genotypes 16/BRBW RES-1, 16/BRBW RES-1, 13/BRBW RES-3, 12/BRBW RES-5, 16/BRBW RES-6, PPL and Arka Kusumakar have distinct differences of genotypic characters and belong at the farthest distances from other genotypes. The remainder of genotypes had similar features forming a separate cluster.

From the plot of principal component and average values selection may be refined considering all 4 principal components, with 16/BRBW RES-2 being the best performing genotype having optimum combination of all variables, followed by genotypes 2012/BRBW RES-5 and 2012/BRBW RES-6 and can be used as improved genetic material for bacterial wilt resistant breeding and other important quantitative traits.

The traits under study exhibited high proportion of GCV to PCV which are under genetic, rather than environmental control, suggesting amenable to selection and predominantly controlled by additive genes. Fruit weight and number were identified as most important selection indices. The pattern of distribution of genotypes from diverse geographical regions into different clusters was random. Host plant resistance has been a major strategy for managing bacterial wilt in brinjal. Breeding for stable resistance is a challenge due to the fact that resistance in brinjal to bacterial wilt can be location-specific, and incorporation of resistance into large-fruited varieties has also been a major challenge to breeders for coming years, suggesting linkage between small fruit size and bacterial wilt resistance. It is concluded from the present assessment that brinjal genotypes showed variations in their resistance response to *R. solanacearum*. None of the cultivars was immune or highly resistant to *R. solanacearum*. Based on D^2 Statistic, PCA and average values three elite

brinjal genotypes 16/BRBW RES-2, 2012/BRBW RES-5 and 2012/BRBW RES-6 possessed optimum combination of variables and could be utilized as donor parents in bacterial wilt disease resistant breeding.

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