Breeding for bacterial wilt disease resistance in brinjal

Abstract

Brinjal is a warm–season vegetable that belongs to the Solanaceae family. Among the biotic stresses of brinjal, bacterial wilt is a major disease caused by a soil-borne bacterium, *Ralstonia solanacearum*. Both cultural and chemical disease control methods are not effective due to the wide host range and prolonged spore survival periods in soil. In recent past, the use of resistant varieties and hybrids are recognized as the effective method to control bacterial wilt disease. Information on genetics of resistance to bacterial wilt disease is of prime importance for improvement of varieties and hybrids in brinjal. The bacterial wilt resistance response and resistance mechanisms are strictly based on the environmental conditions and cultivars. In traditional crop improvement methods, the development of varieties and hybrids is expensive and time consuming. The discovery of molecular marker systems increases the speed and precision for developing of varieties and hybrids with desired agronomic traits.

Key words: Bacterial wilt, Resistance Source, Resistance genetics, Molecular markers

Introduction

"Brinjal (*Solanum melongena* L.) is a warm–season vegetable that belongs to the solanaceae family. It is widely cultivated and freshly consumed in India and other parts of the world. Among the biotic stresses of brinjal, bacterial wilt is a major disease caused by a soil-borne bacterium, *Ralstonia solanacearum*, which reduces the yield from 11.67% to 96.67%, while the humid and congenial climatic condition recorded up to 100% loss". (Bainsla et al., 2016) "The disease was first described by E.F. Smith in potato, tomato and brinjal in 1896 and afterward in tobacco in 1908. *Ralstonia solanacearum* is ranked as the second most destructive pathogen among the 10 most important bacterial species affecting both agricultural and horticultural crops" (Mansfield *et al*., 2012). "This bacterium is responsible for causing disease of more than 200 plant species from 53 different botanical families, including Solanaceous vegetable crops such as potato, tomato, brinjal, chilli and capsicum" (Álvarez *et al*., 2010).

Both cultural and chemical disease control methods such as soil fumigation, crop rotation, adjusting the date of planting and application of chemicals are not effective due to

the wide host range and prolonged spore survival periods in soil. In recent past, the use of resistant varieties and hybrids are recognized as the safest, economical, and effective method to control bacterial wilt disease. Many varieties and hybrids were developed from the public and private sectors are resistant to bacterial wilt disease for many solanaceous vegetable crops.

Information on genetics of resistance to bacterial wilt disease is of prime importance for improvement of varieties and hybrids for resistant to bacterial wilt disease in eggplant. The bacterial wilt resistance response and resistance mechanisms are strictly based on the environmental conditions of the location. The genetics of resistance to bacterial wilt can be described from the segregating populations such as F_2 and back crosses. Many workers have reported that various type of gene actions have been control the bacterial wilt disease resistance in Brinjal. Nevertheless, the genetics of bacterial wilt resistance in brinjal is yet unclear. In resistance breeding, the selection of parents resistant to bacterial wilt is difficult due to environmental factors, such as temperature and pH of the soil that will affect the expression of the disease. These problems can be overcome by identifying closely linked molecular markers to disease resistance loci and using the same in marker-assisted selection (MAS), which helps in rapid identification of the trait of interest in plants and development of durable, resistant cultivars.

Ralstonia solanacearum

The *Ralstonia solanacearum* is a gram–negative, non-spore forming rod shaped bacterium which belongs to the family β–proteobacteria. The optimum temperature for the growth of *Ralstonia solanacearum* was observed between 27–35^{o}C and no growth was observed at above 40^{o}C or below 4^{o}C (Kelman, 1953). The growth of *Ralstonia solanacearum* is optimum in alkaline pH while acidic pH growth is inhibited. The Triphenyl Tetrazolium Chloride (TTC) chemical was used to identify the virulent and avirulent colonies of *Ralstonia solanacearum* strains in the growing medium (Kelman, 1954). The virulent colonies have typical characteristic symptoms of pink or light red color with red center and whitish margin and avirulent colonies are smaller in size, off-white and non–fluidal.

Disease symptoms

The characteristic symptoms of bacterial wilt affected brinjal shows sudden wilt at the time of flowering followed by yellowing of leaves, stunted plant growth, and vascular discoloration. The disease can be easily detected by ooze out test. The slimy ooze was observed from the infected plant by dipping a piece of cut end of stem in a test tube containing sterile distilled water (Ghosh and Mandal 2009). The intensity of the disease depends on the environmental factors such as soil temperature, soil moisture, soil type, soil pH, host susceptibility and virulence of strains.

Bacterial culture isolation

The *Ralstonia solanacearum* infected plants were collected from the bacterial wilt infested sick plot and pathogen was confirmed by ooze out test. The bacterial ooze was observed by dipping a piece of the lower cut stem from just above the root zone in a test tube contain sterile water. After few minutes fine streaks of milky ooze coming out from the margin of the cut portion and the ooze composed of masses of bacteria. The above test distinguishes bacterial wilt from the other wilts like Fusarium wilt and physiological wilt.

The ooze was placed on the sterile agar plates contain Triphenyl Tetrazolium Chloride (TTC), which were incubated for 48 to 72 hours at 30^{o}C (Kelman, 1954). The virulent (irregular shape, white to cream colour, slimy with pink colour in the centre) isolate were selected from the single colony and sub cultured in TTC broth for mass production. After inoculation, the bacterial broth was incubated for 72 hours at 30^{o}C and the bacterial population was adjusted to 0.7 OD at 600 nm wave length (10^{8} cfu/ml). The composition of Triphenyl Tetrazolium Chloride (TTC) media is shown in table 1.

Table 1. Composition of Triphenyl Tetrazolium Chloride media (for 1 litre) at pH 7

S. No.	Chemicals	Quantity
1.	Dextrose	10 g
2.	Peptone	10g
3.	Casein	1 g
4.	Agar	20 g
5.	Distilled Water	1 liter

Artificial inoculation

Thirty days old plants were subjected to artificial inoculation with *Ralstonia solanacearum* culture. The virulent bacterial culture was inoculated by using axil puncturing or pin prick and soil drenching methods. “In axil puncturing or pin prick method, the sterile dissection needle was used for inoculation, the method followed as dipping the needle in the bacterial culture and inserting into the axil of the leaf along with a drop of bacterial inoculum and gently pressing to ensure that the inoculum is reached the vascular tissues. In soil drenching method, the plants roots were little injured with the help of sterile scalpel blade and bacterial culture was drenched 50 mL per plant” (Artal *et al*., 2012).

Resistance test and disease scoring

The disease incidence was recorded and percentage disease incidence (PDI) was calculated for individual germplasm as per Bi-hao *et al*. (2009). The individual germplasm was categorized 0–5 scale (Table 2) based on the PDI value according to Hussain *et al*. (2005). The dead individuals were examined for ooze out test for, in which individuals were died for bacterial wilt or other diseases. Different stages of bacterial wilt disease symptoms in brinjal were shown in Plate 1.

Percentage disease incidence (PDI)

$$\text{PDI} = \frac{\text{Number of wilted plants}}{\text{Total number of plants}} \times 100$$

Table 2. Disease scale for the percentage of wilt plants

Disease response	Percentage of disease incidence
Highly resistant (HR)	Plants did not show any wilt symptom
Resistant (R)	1-20% plants wilted
Moderately resistant (MR)	21-40% plants wilted
Moderately susceptible (MS)	41-60% plants wilted
Susceptible (S)	61-80% plants wilted
Highly susceptible (HS)	More than 80% plants wilted

Resistance sources

The bacterial wilt disease resistance source of brinjal was found in *Solanum torvum* (Gousset *et al*., 2005). Somatic hybrids of *S. melongena* and *Solanum aenthiopicum* produced using protoplast electrofusion and it was found tolerant to bacterial wilt (Collonnier *et al*., 2001). The rootstocks of *Solanum sisymbriifolium* showed resistance against bacterial wilt disease under sick plots in field conditions (Rahman *et al*., 2002). Namisy *et al*. (2019) reported that high levels of resistance were found in *S. sisymbriifolium*, *S. incanum*, *S. anguivi* and *S. torvum* against bacterial wilt disease. The resistance reactions in wild relatives of brinjal and the result shows that species *Solanum ferox* and *Solanum toxicarium* were immune to bacterial wilt disease.

Genetics of resistance

Information on genetics of resistance to bacterial wilt disease is of prime importance for improvement of varieties and hybrids for resistant to bacterial wilt disease in brinjal. The bacterial wilt resistance response and resistance mechanisms are strictly based on the environmental conditions of the location and cultivars. Zhu *et al*. (2004) studied the genetics of resistance in segregated F_2 population of brinjal for bacterial wilt disease. The results revealed that single dominant gene was control the bacterial wilt resistance in brinjal. Gopalakrishnan *et al*. (2005) studied the inheritance of resistance to bacterial wilt disease in brinjal Surya × Pusa Kranti cross. The results of the study suggested that monogenic and incomplete dominance of susceptibility over resistance control the bacterial wilt resistance. Tian *et al*. (2007) studied the genetics of resistance to brinjal bacterial wilt disease in 49 hybrids developed through diallel mating design (4 resistant and 3 susceptible parents). The results were revealed that the genetics of resistance in brinjal bacterial wilt was governed by few recessive genes and influenced by epitasis effect.

Bi–hao *et al*. (2009) studied the genetics of resistance to bacterial wilt disease in F_2s and back cross generations of E–31 (highly resistance) × E–32 (highly susceptible) cross. The results revealed that single dominant gene was governing the resistance in brinjal bacterial wilt disease. The bacterial wilt resistance genetics was studied in four F_2 populations of brinjal which were derived from line × tester design. The bacterial wilt disease incidence was observed and the segregation patterns of four F_2 populations were recorded as 3 (resistance):1 (susceptible) ratio. They identified that the single dominant gene was governing the resistance in brinjal for bacterial wilt disease (Ajjappalavara *et al*., 2010).

Molecular markers for disease resistance

In traditional crop improvement methods, the development of varieties and hybrids is expensive and time consuming. The discovery of molecular marker systems increases the speed and precision for developing of varieties and hybrids with desired agronomic traits. The markers can assist the selection of target alleles, minimize the linkage drag and reduce the number of generations in breeding program. The ideal molecular marker should be highly polymorphic and distributed throughout the genome, require little quantity of genomic DNA, co–dominant give sufficient resolution of genetic differences, generate multiple, linkage to diverse phenotypes and inexpensive (Aggarwal *et al*., 2008). Currently, the majority of DNA markers used for brinjal genetic mapping and resistance breeding are based on PCR, including RAPD, AFLP, SCAR, CAPS, SRAP, SSRs and SNPs.

Lebeau *et al*. (2013) identified the bacterial wilt resistance gene ERs1 in recombinant inbred lines population derived from intra–specific cross between MM738 (Susceptible) × AG91– 25 (resistance) with 4 *Ralstonia solanacearum* strains of phylotype 1. The resistance gene ERs1 is resistant to only 3 strains of phylotype 1 (CMR134, PSS366 and GMI1000) and resistance was broken down with virulent strain PSS4. The action of ERs1 seems to be minimally influenced by environmental factors and mostly dependent on the inoculums strain. Ge *et al*. (2013) assessed 141 brinjal accessions to identify the SSR markers associated with nine fruit traits. The population structure analysis was performed with 105 SSR markers and the results revealed that two subgroups were present in the population and they identified 49 SSR marker associations with 8 fruit traits.

The association mapping for morphological traits of brinjal were done through genome wide association analysis (GWA) with SNP markers. The 194 both phenotype and genotype associations were discovered, related to 30 of the 33 measured traits. The association of 79 SNP marker loci was mapped to 39 distinct chromosomal regions and distributed all 12 brinjal chromosomes (Portis *et al*., 2015). Salgon *et al*. (2018) identified the QTLs resistant to *Ralstonia solanacearum* strains in brinjal mapping population derived from intra–specific cross between MM738 (susceptible) × EG203 (resistant). The 123 doubled haploid lines were used with phylotypes I (PSS4) and III (R3598) strains of *Ralstonia solanacearum.* They identified 10 QTLs with resistant to PSS4 and 3 QTLs resistant to R3598 strains. All the QTLs were strongly influenced by the environment. The most stable QTLs bacterial wilt resistance was identified on chromosomes 3 and 6.

Conclusion

Information on the genetics of resistance to bacterial wilt disease is critical for improving brinjal types and hybrids. The present review well emphasised on Breeding for bacterial wilt disease resistance in brinjal. The climatic circumstances and cultivars have a strong influence on the bacterial wilt resistance response and mechanisms. The discovery of molecular marker systems has increased the speed and precision with which varieties and hybrids with desired agronomic features can be developed.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Competing Interests

Authors have declared that no competing interests exist.

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