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Inheritance of resistance to Bangalore race of *Fusarium* wilt disease in pigeonpea (*Cajanus cajan* L.)

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Abstract

Pigeonpea is one of the important legume crops of India which is affected by *Fusarium* wilt (*Fusarium udum*) disease causing severe yield loss. Four different races of *Fusarium* wilt have reported been with pathogenic race present in Bangalore being most virulent. Hence in the present study nature of inheritance of wilt disease was studied in segregating generations (F_2 and F_3) of crosses namely BRG-1 × ICP-8863 and TTB-7 × ICP-8863. Digenic ratio of 9 (susceptible): 7 (resistant) and 13 (susceptible): 3 (resistant) was obtained in F_2 generation of two crosses BRG-1 × ICP-8863 and TTB-7 × ICP-8863, respectively. Frequency distribution of F_3 generation showed normal curve, skewed towards susceptibility. This indicates that susceptibility was dominant over resistance and is governed by two or more genes. Probable loci responsible for disease reaction have been designated as FuB_1, FuB_2 and FuB_3. Susceptible parents (TTB 7 and BRG 1) shared one common dominant gene whereas ICP 8863 had recessive resistant gene. Characterisation of these genes will help in marker assisted breeding programme.

Keywords: Pigeonpea, *Fusarium* wilt, ICP-8863, F_2 and F_3.
Abbreviations: FuB_*Fusarium udum* Bangalore, S_Susceptible, R_Resistant.

Introduction

Fusarium wilt caused by *Fusarium udum* (Butler) is an important soil borne disease which affects seed yield severely in susceptible varieties. Losses due to wilt disease vary from negligible to complete loss (100%) depending on the stage at which crop is attacked (Kannaiyan and Nene, 1981). The total loss due to wilt disease is approximately 97,000 ton per year in India (Saxena et al., 2002). Soil borne nature of the pathogen makes control of disease by fungicides difficult and is also not eco-friendly. This disease can also be controlled by various crop management practices like pigeonpea-cereal rotation, pigeonpea-tobacco rotation, fallow,green manuring, zinc application, biological control with *Bacillus* (Harish et al., 1998). The utilization of resistant varieties is a classical approach to prevent catastrophic losses caused by wilt disease. It helps to decrease the cost of cultivation and increase production. The search for the source of resistance to wilt in pigeonpea began long back at Pune in India (Butler, 1906). Since then, many workers have screened pigeonpea entries for wilt resistance at various locations. Amongst these entries ICP 8863 reported consistent resistant reaction over years and locations (Nene et al., 1989, Pawar, 1992, Reddy et al., 1995, Mahesh et al., 2005). Thus it was categorized as resistant line for wilt disease. In pigeonpea, resistance to *Fusarium* wilt has been reported to be under the control of two complementary genes (Paramita et al., 2005, Kimani, 1991, Kotresh et al., 2006), single dominant gene (Pawar and Mayee, 1986), two genes (Okiror, 2002), major genes (Paramita et al., 2005, Singh et al., 1998), duplicate genes and even multiple factors (Mehrotra and Ashoka, 2007) and a single recessive gene (Jain and Reddy, 1995). Dominant epistatic gene interaction play a significant role in controlling resistance to wilt (Parimata et al., 2005). These contradictory results could be attributed to variation in experimental methodology as well as screening with different isolates that differ in virulence. Its severity and yield loss vary from place to place due to presence of physiological races in *Fusarium udum* (Patel et al., 2011). Better understanding about the inheritance of wilt resistance for Bangalore race was required to develop resistant cultivars. Present study was aimed at understanding the inheritance of resistance to *Fusarium* wilt for Bangalore race using ICP 8863 as a donor parent.

Results

Three pigeonpea genotypes viz., BRG 1, TTB 7 and ICP 8863 selected as parents based on the previous screening reports for wilt resistance were confirmed for their resistance during the present study. The resistant parent ICP 8863 showed 100 per cent resistance to wilt disease while, the susceptible genotypes TTB 7 and BRG 1 showed 100 per cent wilt susceptible reaction (Table 1).

Table 1. Performance of parents to Fusarium wilt grown under sick plot condition.

Sl. No	Material	No. of Plants	Resistant plants	Susceptible plants	Disease incidence (%)	Disease reaction
Parents						
1	TTB 7	15	0	15	100	Susceptible
2	BRG 1	15	0	15	100	Susceptible
3	ICP 8863	15	15	0	0	Resistant

Table 2. Reaction of F_2 generation to Fusarium wilt grown under wilt sick plot.

Cross		No. of plants	Observed frequency		Expected frequency		Ratio S:R	χ^2 value (cal)	χ^2 value (Tab)
			Resistant	Susceptible	Resistant	Susceptible			
BRG1 ICP8863	×	94	43.00	51.00	41.16	52.92	9:7	0.15	3.84
TTB7 ICP8863	×	160	41.00	119.00	48.00	112.00	13:3	1.46	3.84

Table 3. Descriptive statistics of Fusarium Wilt reaction in F_3 generation in different crosses.

Cross	Progeny row	Mean	Range	Standard deviation	Standard error	Skewness	Kurtosis
BRG1 × ICP8863	114	33.68	0-100	29.41	2.75	0.76	0.013
TTB7 × ICP8863	185	21.55	0-100	23.22	1.71	1.20	1.15

Screening of F_2 generation

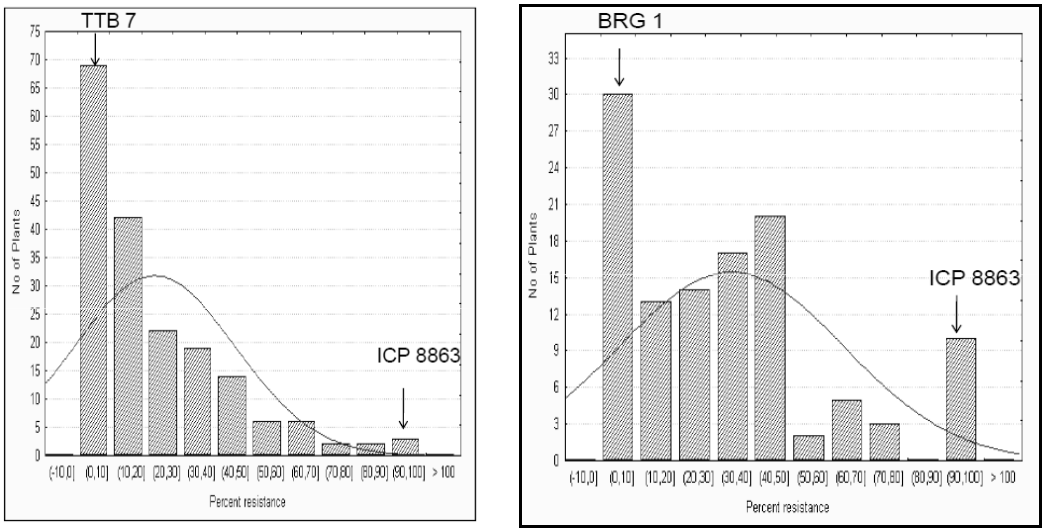
Segregation pattern for *Fusarium* wilt resistance among cross BRG1 × ICP8863 and TTB 7 × ICP 8863 are given in Fig 2 and Fig 3. In the cross BRG 1 × ICP 8863 susceptible parents had two dominant alleles whereas resistant parent had recessive alleles for both loci. Proposed allelic combinations of susceptible parents BRG 1 and TTB 7 are *FuB1FuB1FuB2FuB2* and *FuB3FuB3FuB2FuB2* respectively. The $_{F1}$ was found to be susceptible in both crosses. All F_2 plants from both crosses were grouped into two classes viz., resistant with no wilting symptoms and susceptible with severe wilting symptoms. In the cross BRG 1 × ICP 8863, out of 94 F_2 plants screened against wilt, 43 plants were resistant and 51 plants were susceptible (Table 4) indicating the segregation ratio of 9 (susceptible): 7 (resistant). In cross TTB 7 × ICP 8863, out of 160 plants screened in F_2, 41 plants recorded resistant reaction while remaining 119 plants were susceptible (Table 4) resulting in the segregation ratio of 13 (susceptible): 3(resistant). The homogeneity chi-square value was within the acceptable limit for both the crosses resulting in a good fit for the expected 9:7 and 13:3 segregation ratios respectively in both the crosses.

Screening of F_3 generation

Descriptive statistics of *Fusarium* wilt incidence in F_3 generation of two crosses is given in Table 3. Wilt incidence in both the crosses ranged from 0 to 100 per cent with skewness of 0.762 in the cross BRG 1 × ICP 8863 and 1.202 in TTB 7 × ICP 8863 cross. Kurtosis values were -0.013 for BRG 1 × ICP 8863 cross and 1.147 for TTB 7 × ICP 8863. The variation existing in F_3 populations for wilt was represented graphically (Fig 1) using frequency distribution of means for two crosses. The disease scores were plotted on X-axis against genotype frequency on Y-axis with equal class interval. The resulting histogram showed near normal curves for both the crosses skewed towards susceptible parent. The distribution was within the parental limits for both the crosses.

Discussion

A basic knowledge of number of genes controlling resistance and their inheritance pattern will help to design efficient breeding programmes to develop resistant varieties. Earlier reports have revealed that resistance to wilt disease was controlled by multiple factors (Pal, 1934), complementary genes (Shaw, 1936; Pathak, 1970), duplicate genes (Joshi, 1957) and single dominant gene (Pawar and Mayee, 1986). The present study was carried out to understand the genetics of *Fusarium* wilt resistance by crossing resistant line ICP-8863 with susceptible lines TTB 7 and BRG 1. The segregation pattern in F_2 generations of susceptible × resistant crosses revealed digenic ratio of 9 susceptible: 7 resistance for cross BRG 1 × ICP 8863 and 13 susceptible: 3 resistant for the cross TTB 7 × ICP 8863. Thus, indicating the complementary gene actions in BRG 1 × ICP 8863 cross and inhibitory gene action in TTB 7 × ICP 8863 cross. Similar segregation ratios of 9 (susceptible): 7 (resistant) and 13 (susceptible): 3 (resistant) were observed by using ICP 8863 as a donor parent by Odeny (2000) and Odeny et al., (2009b). Complementary gene action (9:7) in the cross BRG 1 × ICP 8863 is probably due to segregation of genes 'FuB_1' and 'FuB_2' (Fig. 2). Resistance to wilt disease is expressed when one of the genes at a locus is in homozygous recessive condition ($FUB_1\_fub_2\ fub_2$) or both the genes are in homozygous recessive condition ($fub_1fub_1fub_2fub_2$). On the contrary, susceptible reaction is expressed when both the dominant genes are present ($FuB1\_FuB2\_$). In case of inhibitory gene action (13:3) in cross TTB 7 × ICP 8863 (Fig. 3), one of the two loci had inhibitory effect on the other loci (FuB_3). Resistant reaction is expressed in the absence of recessive inhibitory gene at one locus ($FuB_3\_fub_2fub_2$). All the other combinations will give susceptible reaction. The pattern of frequency distribution for wilt disease incidence in F_3 generation in two crosses showed continuous distribution pattern indicating that wilt disease is controlled by more than two genes. However, large number of plants could be classified into susceptible and moderately susceptible groups.



a) TTB 7 × ICP 8863 b) BRG 1 × ICP 8863

Fig 1. Frequency distribution of *Fusarium* wilt incidence in F_3 Generation of a) TTB 7× ICP-8863 and b) BRG 1 × ICP 8863.

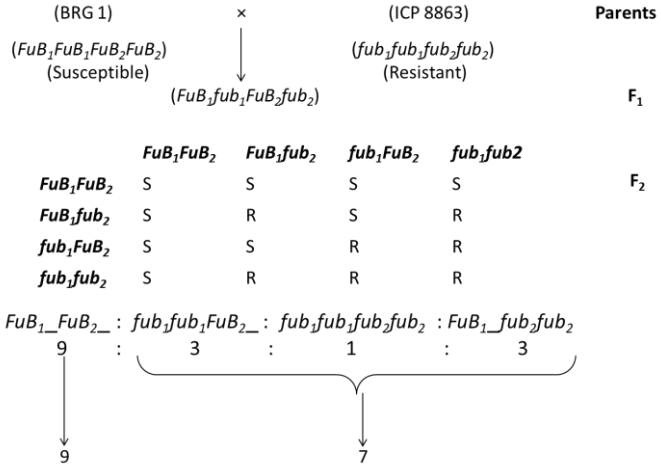


Fig 2. Schematic representation of segregation behaviour of FuB_1 and FuB_2 genes in the cross BRG-1 × ICP-8863. FuB_1 and FuB_2 are dominant alleles for loci *Fusarium udum* Bangalore (*FuB*) 1 and 2 respectively and fub_1 and fub_2 are recessive alleles. "_" indicates presence of either dominant or recessive allele. "S" = Susceptible and "R" = Resistant.

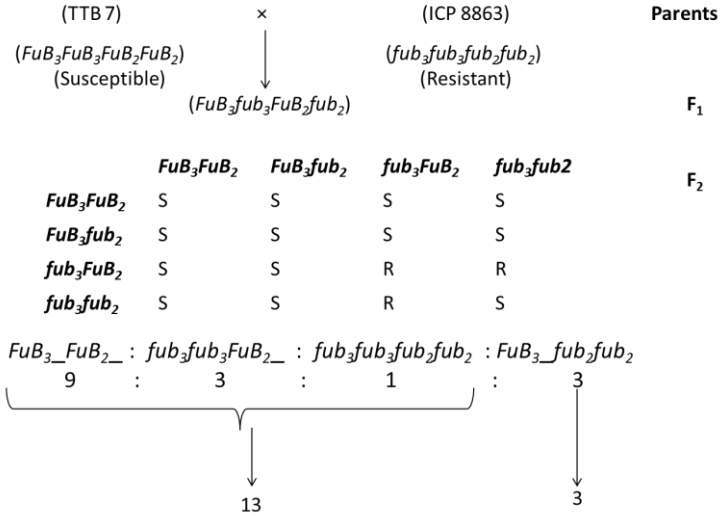


Fig 3. Schematic representation of segregation behaviour of *FuB1* and *FuB2* genes in the cross TTB-7 × ICP-8863. FuB_3 and *FuB2* are dominant alleles for loci *Fusarium udum* Bangalore (*FuB*) 1 and 2 respectively and fub_1 and fub_2 are recessive alleles. "_" indicates presence of either dominant or recessive allele. "S" = Susceptible and "R" = Resistant.

Only few plants were classified into resistant group. Frequency distribution of wilt disease was positively skewed (towards susceptibility) indicating that susceptibility was dominant over resistance. In the present study, one of the genes from BRG-1 had complementary effect while gene from TTB 7 had inhibitory effect on the resistant gene from ICP 8863. Hence two female parents in the present study namely TTB 7 and BRG 1 share one common dominant gene (FuB_2). ICP 8863 is contributing one recessive resistant gene (fub_2) for which other two parents have common dominant gene (FuB_2). Considering the results of these two crosses, resistance to *Fusarium udum* in pigeonpea is governed by two or more genes. A similar observation of multiple gene action for *Fusarium* wilt resistance in pigeonpea was reported by Joshi (1957). Characterisation of these wilt resistance genes will help in developing gene specific markers which could be utilised in marker assisted breeding programme.

Materials and methods

The material for the study comprised of three diverse genotypes selected based on previous reports for their resistance levels. The resistant parent ICP-8863 differed from susceptible parents TTB 7 and BRG 1 with respect to high level of intrinsic resistance to *Fusarium* wilt. Populations were developed at All India Coordinated Research Project on Pigeonpea, University of Agricultural sciences, Bangalore by crossing susceptible parents (TTB 7 and BRG 1) with resistant parents (ICP 8863). Hybridization was carried out during March, 2008 under insect proof nylon net to prevent natural out-crossing to produce sufficient F_1 seeds.

Raising of segregating generation

Morphological traits such as plant type, flower colour and pod colour were used as markers to check the trueness of F_1 plants. F_0 generation obtained after crossing was divided into two parts. One part of F_0 generation was sown during July 2008 to raise F_1 generation whereas second part was saved. F_1 generation obtained after harvest was again divided into two parts. One part was sown to raise F_2 generation whereas second part was saved for future use. Each plant in F_2 generation was harvested individually to raise F_3 generation during July 2009. Parents and F_1 generation consisted of 15 plants in each replication whereas F_2 generation consisted of 94 plants in the cross BRG 1 × ICP 8863 and 160 plants in the cross TTB 7 × ICP 8863. F3 generation of cross BRG 1 × ICP 8863 consisted of 114 progeny rows, whereas cross TTB 7 × ICP 8863 consisted of 185 progeny rows and each progeny rows consisted of 10 plants.

Preparation of giant culture of Fusarium udum isolate

Giant culture of *Fusarium udum* was prepared in the proportion of 95:5 w/w sand and maize meal mixtures and moistened with sterile water to 20 per cent of volume in order to get maximum inoculums of *Fusarium udum*. About 500g of mixture was taken in 1000 ml conical flask and were sterilized at 15lb pressure at 121°C for 20 minutes. These flasks were inoculated with the culture of *Fusarium udum* isolates under aseptic condition and incubated at a temperature of 28±1°C for 30 days. The flasks were shaken every day to get uniform growth of culture. The giant culture so obtained was used for screening parents and segregating population.

Screening parents and segregating populations for Fusarium wilt resistance

Giant culture was thoroughly mixed with autoclaved soils separately at 1:4 w/w ratios. The mixture was filled in 15 cm diameter polythene bag. Ten seeds per family of F_3 population (BRG 1 × ICP 8863 and TTB 7 × ICP 8863) were sown during July 2009 in polybags along with parents and F_1's in two replications, whereas F_2 was sown without replication. Plants grown in polybags were watered regularly so as to maintain 50 per cent water holding capacity of soil. Plants of parents, F_1's, F_2's and F_3's that wilted at maturity were classified as susceptible and those which did not wilt were recorded as resistant. Plants were classified as resistant (no wilting symptoms) or susceptible (wilting symptoms). The goodness of fit to Mendelian segregation of resistant and susceptible plants in the segregating population was tested by Chi- square test (χ^2) (Pearson, 1922) where $\chi^2 = (o_i - e_i)^2 / e_i$.
Where,
oi = Observed frequency of plants
ei = Expected frequency of plants
The significance of chi- square value was tested against table value with (n-1) degrees of freedom, where 'n' is the total number of segregating classes.

Conclusion

Results of present study indicated that resistance to *Fusarium* wilt in pigeonpea is governed by more than two genes. Susceptible parents shared one common gene i.e FuB_2 and differed for other genes. Previous reports have indicated that number of genes controlling resistance depend on parent material used as it is evident in the present in the present study. This knowledge of genetics of *Fusarium* wilt resistance could be used to breed resistant high yielding cultivars. Characterisation of these genes will help in marker assisted breeding programme.

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