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Genetics of *fusarium wilt* resistance in pigeonpea as revealed by phenotyping of RILs

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ABSTRACT

***Fusarium wilt* is one of the most destructive biotic stresses of pigeonpea [*Cajanus cajan* (L.) Millsp.] and the annual production losses approach over US $71 million. Availability of high yielding wilt resistant cultivars with wide adaptation is the ideal solution to overcome this production constraint on a sustainable basis. To achieve this, a clear understanding of the genetic control of disease resistance helps in formulating appropriate breeding strategies. Hence, the present study was undertaken to determine the mode of inheritance of FW using mapping populations, including two sets of Recombinant Inbred Lines (RILs). The phenol typing data revealed that the resistance to FW was controlled by mongenic dominant and recessive gene actions in the two RILs. Besides this, the breeding materials generated from this study can be used to derive high-yielding inbred cultivars and hybrid parents.**

Keywords: *Fusarium wilt*, Gene action, Inbred cultivars, Pigeonpea, Resistance, RIL

Enhancing population at a regular pace in Indian sub-continent necessitates the quest for increasing crop production to fulfil the issues related to wide spread mal-nutrition. Despite various research and extension efforts in this part of the world, there persists a huge gap between demand and supply of protein-rich food. For example in India, the per capita protein requirement has reduced from 66 g/day/head in 1965, to <33g/day/head in 2005 (Tomar and Talukdar, 2016). Pulses are the cheapest source of protein to the poor people, and within this group of crops, pigeonpea (*Cajanus cajan* (L.) Millsp.) is playing an important role because of its nutritious grains (Saxena *et al.* 2010) and ability to produce reasonably good yields even in marginal lands and under stressed environments (Saxena, 2008). Globally, the cropped area of pigeonpea stands around 7 mha in tropical and sub-tropical regions; and India is the undisputed leader, accounting for 80% of global area (7 mha) and 67% of the global (4.9 mt) production (FAO, 2017). Stability of production is, however, a serious constraint in this crop; and various biotic (diseases and insects) and abiotic stresses (drought, water-logging, salinity) are the major yield reducers.

With regards to diseases, pigeonpea is known to be attacked by more than 60 different pathogens (Reddy *et al.* 2012); but only a few of them are destructive. These include FW, sterility mosaic disease and Phytophthora blight. Among these, FW caused by *Fusariu mudum* Butler, is the most devastating with annual economic losses approaching as high as US $71 million (Sharma *et al.* 2013). This fungus can survive in field for up to five years and in the infected plants it impairs their vascular system and results in partial or complete wilting (Reddy *et al.* 1993). Deployment of high yielding cultivars with genetic resistance is the most effective way to overcome this production constraint. Breeding for disease resistant cultivars has three basic requisites including (i) availability of pure genetic stocks with high levels of genetic resistance, (ii) understanding the genetic nature of genes controlling resistance, and (iii) effective disease screening technology. At present, information on the genetic control of resistance to FW disease is inconclusive; therefore, to understand the inheritance patterns of resistance to FW, the present study was undertaken, using two sets of recombinant inbred lines (RILs).

MATERIALS AND METHODS

Development of RILs: Two hybrid combinations were made by crossing inbred lines with contrasting disease reaction (Table 1). The first hybrid (PRIL-A) was developed from a cross involving susceptible ('ICPB 2049') and resistant ('ICPL 99050') lines; while the second hybrid (PRIL-B) was developed by crossing resistant genotype ('ICPL 20096') with susceptible ('ICPL 332') genotype. In 2007 rainy season the crossed seeds were sown in field and F_1 hybrid plants were selfed to advance the generation by growing them under a large nylon net to prevent cross pollination. From each F_2 population,188 plants were selected randomly without any selection and further generation advancements were done following single seed descent method (Varshney *et al.* 2010) until the breeding materials reached the homozygous (F_7) stage.

Phenotyping of RILs: Phenotyping of both RIL sets for FW resistance was performed against 'Patancheru isolate'

of *Fusarium udum* in a wilt-sick nursery at ICRISAT, Patancheru. A total of 188 RILs from each cross were evaluated in two replications. Thirty seeds representing each RIL were sown in four replications in the second week of July 2014 with inter- and intra-row spacing of 75 cm and 10 cm, respectively. To monitor the disease spread/ incidence in the sick nursery, single row of the FW resistant ('ICP 8863') and FW susceptible ('ICP 2367') inbreeds were planted after every 10 rows of the test materials as disease monitoring checks. Recommended cultural practices were provided to raise a healthy crop. The stand in each plot was satisfactory (>0 plants/row). The plants in the susceptible check rows started wilting from 30 days onwards after sowing; and phenotyping of the progenies with respect to mortality due to FW was carried out by a Pulse Pathologist, 90 days after sowing, when the susceptible check rows had 95 - 100% wilted plants. The RIL populations were scored visually on single plant basis for FW disease symptoms according to the methodology proposed by Singh *et al*. (2003) and expressed as per cent disease incidence (PDI). For studying the inheritance of the disease resistance, the progenies with 0 to 20 PDI were considered 'resistant'; and those with >20% as 'susceptible'; and mean PDI from the two replication data was subjected to chi-square (+2) test for estimating the number and nature of genes controlling the expression of FW resistance.

RESULTS AND DISCUSSION

Screening of pigeonpea germplasm of primary gene pool revealed the presence of a number of FW resistance sources and it represented a vast geographic diversity (Sharma *et al.* 2011, 2012 and 2013). However, in order to develop promising genetic materials combining wilt resistance and higher yields, it is important that information on the inheritance is available to the breeders. In this context, a number of studies have been conducted in the past using different FW resistant and susceptible parents. Screening of the genetic materials generated from PRIL-A ['ICPB 2049' × 'ICPL 99050'] revealed that out of 376 progenies tested, 281 were susceptible and 95 resistant. Overall, the PRIL-A progenies segregated in to the expected ratio of 3 susceptible: 1 resistant (P= 0.90). The 3 susceptible: 1 resistant ratio obtained indicated that, susceptibility was dominating in the population and FW resistance in 'ICPB 2049' was under the control of a single recessive gene pair. In PRIL-B '[ICPL 20096' × 'ICPL 332'], screening the screening of a total of 376 progenies revealed 280 resistant and 96 susceptible progenies and it fit well to the expected ratio of 3 resistant: 1 susceptible (P=0.81). This segregation pattern suggested that FW resistancein PRIL-Bwas controlled by a single dominant gene, contributed by the resistant parent, 'ICPL 20096'. The identified major genes and their gene actions contributing to FW resistance could be potential resources for resistance breeding.

Screening of the RILs for FW resistance yielded contrasting results with respect to inheritance of the resistance genes. In one case it was single recessive while in the other a dominance gene was detected. These different genetic systems with regards to FW resistance can be due to the use of two different resistance sources in developing PRIL-A and PRIL-B. In parent 'ICPL 99050', FW was controlled by single recessive gene, while in 'ICPL 20096' it was controlled by a dominant gene. In order to get more insight on the genetic nature of FW resistance, we compared the present results with that of published information. The review of literature revealed a lot of variation, both in terms of number of genes and their mode of action in controlling the expression of resistance. The resistance to FW was found to be controlled by single recessive gene in studies conducted by Jain and Reddy, 1995; Karimi *et al.*2010; Patil *et al.* 2013). In contrast, Pande *et al.* (1996), Kotresh *et al.* (2006), Chaitanya *et al.* (2011), and Changaya *et al.* (2012) reported that the resistance to FW was under the control of single dominant gene. The involvement of two genes in governing the resistance were also reported but with different gene actions. For instance, Odeny *et al.* (2009), Ajay *et al.* (2013), and Singh *et al.* (2016) found that the FW resistance in pigeonpea was controlled by two dominant with complimentary gene action. In some other studies, two genes with inhibitory action were reported (Okiror, 2002, Kumar *et al.* 2009, and Saxena *et al.* 2012). Besides all these, Changaya *et al.* (2012), Patil *et al.* (2013), and Singh *et al.* (2016) also reported that the two genes that controlled the resistance reaction were inherited as duplicate dominant genes.

This is a complex situation to explain as far as inheritance of FW resistance is concerned, and we assume that such a large variation in the results of different studies could be attributed to the factors such as (i) differences in the genetic constitution of the parents involved in the study, (ii) presence of different biotypes of the pathogen at different experimental sites, (iii) variation in the intensity of active inoculum in the screening nursery, (iv) different soil environmental conditions, (v) the previous crop grown in

Table 1. Wilt reactions of parental lines and their crosses

Genotype	Material	Parentage	Wilt (%)
'ICPB 2049'	Advance breeding line	'ICPA 2039' × 'ICP 6697'	90-100
'ICPL 332'	Advance breeding line	'ICP 1903' selection	90-100
'ICPL 99050'	Advance breeding line	'C11' × 'ICP 8863'	00-90
'ICPL 20096'	Advance breeding line	'ICPL 87119' × 'ICP 12746'	00-90
Single cross	RIL-A	'ICPB 2049'(S) × 'ICPL 99050'(R)	00-100
Single cross	RIL-B	'ICPL 20096'(R) × 'ICPL 332(S)'	00-98

the field, and (v) growth stage and method of scoring the plants for disease incidence. The present study was conducted with the objective of deciphering inheritance of wilt resistance, using two sets of RILs. This genetic material, through successive recombination/ segregation events for five generations with no selection pressure, achieved homozygosity to draw logical conclusions about the genetic control of FW disease. The replicated evaluation of the disease reaction through a large population *i.e.* 120 plants/ progeny and nearly 22,000 plants/ RIL) should have allowed neutralization of environment effect to a great extent on the phenotypic expression of the trait, which could be deployed for the effective trait mapping (Alonso-Blanco *et al.* 1998). Heritability of a trait is a prominent tool in achieving genetic advance in breeding. The estimates of the heritability are more profound in RILs as compared to early generation mapping population (Takuno *et al.* 2012).

Okiror (2002) considered the multi-genic resistance to be more comprehensive and advantageous over monogenic resistance with respect to its durability. Considering the losses caused by FW disease in pigeonpea and the persistence of the fungus under field conditions, it is imperative to develop pigeonpea varieties that have durable resistance across diverse environments. For breeders it is important that a right choice is made in selecting the source of resistance for hybridization. Preference should be given for lines with good agronomic base, high combining ability, dominant multiple gene action, and resistance to other diseases. In pigeonpea, the hybrid breeding is a recent innovation with high yield gains (Saxena *et al.* 2018). In this breeding programme also, 'ICPL 20096' can be used to produce wilt resistant hybrids with ease. Mudaraddi and Saxena, (2015) found that 'ICPL 20096' fully restores the pollen fertility of A_4 CMS system. The frequency of resistant hybrids made on a range of CMS lines will be higher if 'ICPL 20096' is used as male parent. It is simply because all the hybrids made by crossing 'ICPL 20096' with a susceptible or resistant female (A-line) parent will produce resistant hybrids. In contrast for producing resistant hybrids using a restorer line with recessive genetic control, both the parents should have similar recessive control of the disease. The present study revealed the number and action of genes conferring resistance to FW, but additional mapping studies could be undertaken to identify the genomic regions involved in imparting the resistance. The RILs used in this study are ideal resource for conducting QTL analysis, which will allow precise identification of the FW resistant genes. In addition, these RILs could also be used to examine the effects of different biotypes (races) of *Fusarium udum* on the expression of wilt disease.

The current study also indicated that during the development of RILs, the genetic variability with respect to FW was more or less conserved and that helped in revealing the genetics of resistance to FW. The uniformity recorded within the progenies indicated that the homozygosity achieved through single seed descent method was very high and it will allow breeders to select for other key traits among the resistant progenies. In breeding programme, 'ICPL 20096' where a dominant gene controlled the resistance appears to be a better choice than 'ICPL 20096' because the former will yield greater proportion of resistant progenies and hence provide greater choice to breeder for exercising selection.

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