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Genetic variability for fusarium wilt disease reactions in pigeonpea (*Cajanus cajan* L.) Millsp.

Hemanth Byatroy, IS Katageri and Basavaraj Bagewadi

Abstract

Pigeonpea is India's most significant pulse crop. The principal biotic restrictions in pigeonpea production is fusarium wilt (FW). To overcome this, genomics-assisted breeding (GAB) is essential to develop varieties which are not only high yielding but also resistant to FW. In this study, 308 recombinant inbred lines (RILs) developed for FW resistance (GS 1 X MARUTI designated as RIL 1) were screened at Agriculture research station, Gulbarga in *kharif* season 2016-17 for fusarium wilt disease reactions. Among 308 RILs, 103 RILs were found to express susceptible disease reaction with FWDI of 30 to 100%, 102 RILs were found to express moderately resistance disease reaction with FWDI ranging from 10.1 to 30% and 103 RILs were found to be expressing resistant disease reaction with FWDI ranging from 0 to 10%. Overall, it was found that the genotypes showed an average of 29.5% FWDI with high broad sense heritability, GCV and PCV. This study is a stepping stone to identify resistant lines and also to develop high density genetic map and identify QTLs for FW disease reactions.

Keywords: Pigeonpea, *fusarium udum*, fusarium wilt disease incidence, PCV, GCV

Introduction

In India, pigeonpea (*Cajanus cajan* L.) is known as *tur* or *arhar*. Pigeonpea is a perennial legume crop grown in tropics and subtropics that belongs to Fabaceae family (Vanaja *et al.*, 2010) [10]. Pigeonpea is a tropical and subtropical plant with a deep taproot system, it is also heat tolerant, and has a robust growth habit that makes it ideal for rainfed agriculture in semi-arid regions (Mallikarjuna *et al.*, 2011) [3]. It is the sixth most significant grain legume crop planted throughout Asia, Africa, and the Caribbean under a range of cropping techniques (Mula and Saxena, 2010) [5]. India is the world's second-largest producer of pulses, accounting for over 90% of global production. Pigeonpea is cultivated on 4.42 million hectares in India, with an annual yield of 2.89 million tonnes and a productivity of 655 kg ha⁻¹. India has the highest land area (3.38 million hectares), followed by Myanmar (580,000 hectares), China (150,000 hectares), and Nepal (150,000 hectares) (Japur *et al.*, 2016) [12].

Pigeonpea's poor yield is caused by a variety of diseases, insect pests, drought, and heat stress, among other things. One serious disease which affects the yield is Fusarium wilt (FW) caused by fungus *Fusarium udum* (Butler). This is also true for India and African countries such as Kenya, Tanzania, and Malawi (Singh *et al.*, 2016) [8]. In India, FW is said to have caused yield losses of USD 71 million in 2011, with losses of USD 5 million in eastern Africa (Reddy *et al.*, 2012) [7]. FW is a destructive pigeonpea disease that is rising in significance as the country's drought circumstances worsen. As the fungus is soil borne in nature and can lay dormant for a longtime. Chemical management of this disease is not only costly, but also ineffective. As a result, there is an urgent need to develop varieties that can overcome this constraint (Ravuri *et al.*, 2013) [6].

Pigeonpea has a lot of genetic diversity owing to its nature as it is an oftenly cross-pollinated crop, which enables it to be grown in a variety of settings and cropping methods, including solo and intercropping (Deepti *et al.*, 2021) [11]. For pigeonpea production to be sustainable, high-yielding cultivars must be created, and the crop must be safeguarded against numerous biotic and abiotic yield stresses. Genetic parameters such as genotypic and phenotypic coefficients of variation, heritability, and genetic advance are extremely reliable for selecting breeding materials. This study was conducted to screen the pigeonpea RIL population and assess phenotypic variability (PV), genotypic variability (GV), heritability in a broad sense (h²S), and genetic advance (GAM) for fusarium wilt disease reactions. This information is extremely helpful for breeders in selecting breeding lines for the development of resistant lines. (SNCVL *et al.*, 2018) [9].

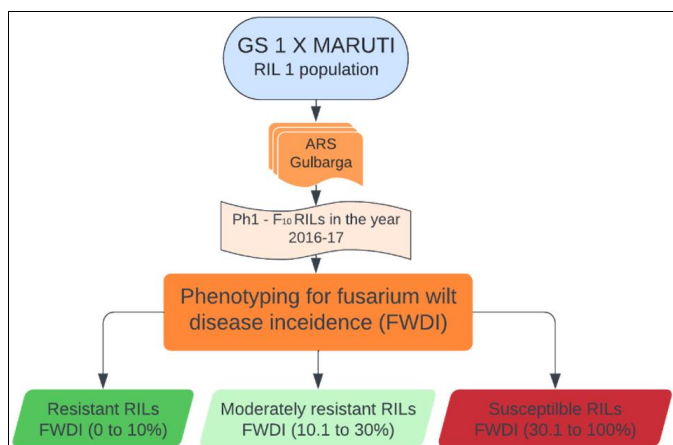


Fig 1: A schematic representation of various steps performed in the present study

Materials and methods

A recombinant inbred line (RIL) mapping population derived from the cross between (GS 1 X MARUTI designated as RIL 1). This population was specifically designed to identify high yielding and fusarium wilt resistant RILs. RIL 1 was screened for fusarium wilt in wilt sick plot of Agricultural Research Station (ARS), Gulbarga, Karnataka, India in 2016-17 *kharif* season. A total of 308 RILs were used for this study along with three checks *viz.*, Maruti (resistant parent), BSMR 736 (resistant check) and ICP 2376 (susceptible check). These were planted through hand sowing in augmented block design, which contained beds of 3 m and distance between the beds were 1 m, the seeds were sown with 30 cm distance between rows and 10 cm distance between plants in 3 m long row. The depth of sowing was 4-5 cm on a ridge and furrow bed system. Phenotypic observations were scored for fusarium wilt disease incidence (FWDI) at three distinct stages of the crop's development, which correspond to the seedling, mid-stage, and late-stage stages of development, respectively as per disease rating scale for fusarium wilt by All India Co-ordinated Research project on pigeon pea (AICRP). Individual lines were evaluated based on their ability to withstand the symptoms of wilt in the field. This was calculated by the percent of fusarium disease incidence (FWDI) in a line, a line was considered as resistant if it showed 0 to 10% of FWDI; if a line shows 10.1 to 30% of FWDI, then it was considered as moderately resistant; if the FWDI was more than 30% then that RILs were classified as susceptible. Before analysis, FWDI data were transformed using arcsine transformation so that residual terms would have a normal distribution. (Warton and Hui, 2011) [11].

$$FWDI (\%) = \frac{\text{Total number of infected plants in a line}}{\text{Total number of plants in a line}} \times 100$$

For statistical analysis, the data was subjected to RStudio version 2022.02.3 for calculation of genetic variability (GV), phenotypic variability (PV), broad sense heritability (h^2_{bs}), genotypic coefficient of variation (GCV), phenotypic

coefficient of variation (PCV), environmental coefficient of variation (ECV), genetic advance (GA) and genetic advance over mean (GAM), It's not a routine to do these analysis for disease incidence traits, however similar studies have been done in other crops and have found to be invaluable (Mubai *et al.*, 2019) [4].

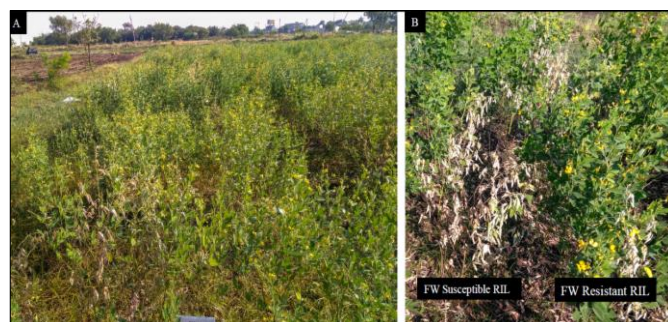


Fig 2: A) Field view of RIL 1 population in fusarium wilt sick plot of ARS, Gulbarga in 2016-17 for FW screening. B) RILs showing FWDI.

Results and Discussions

In 2016-17 *kharif* season, 308 lines of RIL 1 population were screened for fusarium wilt disease incidence (FWDI) in ARS, Gulbarga. Among the 308 RILs that were screened, 103 RILs were found to be resistant, with FWDI ranging from 0 to 10%; 102 RILs were found to be moderately resistant, with FWDI ranging from 10.01 to 30%; and the remaining 103 RILs found to be susceptible, with FWDI ranging from 30 to 100% these are represented in Table 1 and Appendix I. The diversity of genetic variations seen in FWDI data suggested that several genes are involved in fusarium wilt disease reactions making this population suitable for further studies especially as Quantitative trait loci (QTLs) identification.

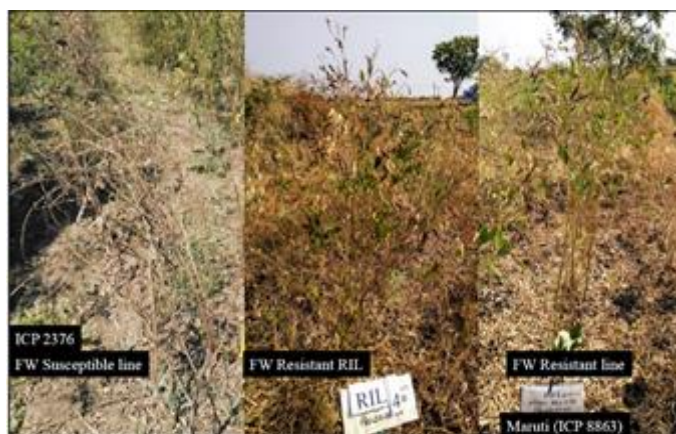


Fig 3: RILs of RIL 1 population showing FWDI in ARS, Gulbarga.

Table 1: FWDI screening in RIL 1 population.

Disease reactions	Number of RILs
Resistant (0-10%)	103
Moderately resistance (10.1-30%)	102
Susceptible (30.1-100%)	103
Total Screened	308

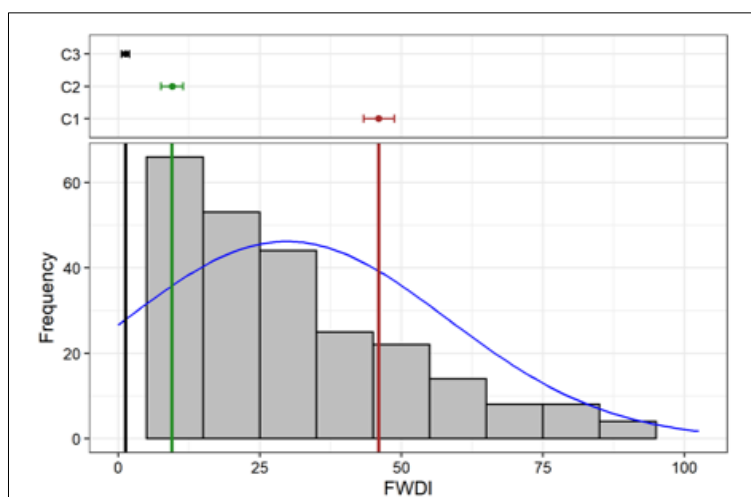


Fig 4: Frequency distribution table showing wide range of variations in RIL 1 (FW) Population; C1- ICP 2376, C2- Maruti and C3- BSMR 736

Analysis of variance revealed that there were significant differences between the lines for FWDI. The mean, standard deviation, range and other genetic parameters such as genotypic and phenotypic coefficients of variation (GCV and PCV), heritability (h^2S), and genetic advance over mean (GAM) are presented in Table 2. Mean for the FWDI was found to be 29.5, observations ranged from 0 to 100%. PCV and GCV were found to be high compared to ECV indicating the expression of disease reactions is not influenced by environment.

Table 2: Genetic variabilities for FWDI trait of RIL 1 Population.

Genetic variabilities	FWDI
Mean	29.56
Range	0-100
S. D.	28.4
PV	541.9
GV	497.5
EV	44.43
GCV (%)	112.5
PCV (%)	117.4
ECV	33.6
h^2bs (%)	91.8
GA	44.09
GAM	222.3

h^2bs (%): Heritability (broad sense), GV: Genetic variability, PV: Phenotypic Variability, GCV: Genotypic coefficient of variation, PCV: Phenotypic coefficient of variation, ECV: Environmental coefficient of variation, SD: Standard deviation, GA: Genetic advance, GAM: Genetic advance over mean.

Conclusion

This study assisted in the identification of 103 RILs resistant to FW, of which 56 lines exhibited FWDI values ranging from 0% to 5%, making them ideal for the selection of disease-resistant varieties. In addition, the study revealed a high level of genetic variability for the FWDI trait evaluated in RILs, which can be utilized in pigeonpea breeding programmes, and the data obtained from this study can be used to identify QTLs for fusarium wilt disease reactions.

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Ethical Matters

Not Applicable, there was no usage of experimental animals and human subjects in these research article. Hence, there is no need to seek approval from any committee.

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Appendix

Appendix I: Screening of GS 1 X MARUTI RIL population (FW) for Fusarium wilt disease at ARS, Gulbarga (2016-17)

RILs	FWDI (%)	Disease reaction	Transformed FWDI
1	21.4	MR	12.4
3	0.0	R	0.0
4	0.0	R	0.0
7	9.1	R	5.2
9	37.5	S	22.0
10	25.0	MR	14.5
11	0.0	R	0.0
12	62.5	S	38.7
13	64.3	S	40.0
14	40.0	S	23.6
15	33.3	S	19.5
16	31.8	S	18.6
17	35.7	S	20.9
18	14.3	MR	8.2
20	17.6	MR	10.2
21	0.0	R	0.0
22	42.9	S	25.4
23	21.4	MR	12.4
24	25.0	MR	14.5
26	0.0	R	0.0
28	9.1	R	5.2
29	0.0	R	0.0
30	16.7	MR	9.6
31	19.0	MR	11.0
32	0.0	R	0.0
33	0.0	R	0.0
35	9.1	R	5.2
36	10.0	R	5.7
38	0.0	R	0.0
39	13.3	MR	7.7
40	44.4	S	26.4
41	56.3	S	34.2
42	33.3	S	19.5
43	7.1	R	4.1
44	7.7	R	4.4
45	5.3	R	3.0
46	0.0	R	0.0
47	0.0	R	0.0
48	0.0	R	0.0
49	3.8	R	2.2
50	0.0	R	0.0
51	21.4	MR	12.4
52	17.6	MR	10.2
53	33.3	S	19.5
54	22.2	MR	12.8
55	25.0	MR	14.5
56	0.0	R	0.0
57	80.0	S	53.1
58	50.0	S	30.0
59	56.0	S	34.1
60	52.6	S	31.8
61	26.3	MR	15.3
62	33.3	S	19.5
63	47.6	S	28.4
64	28.6	MR	16.6

65	5.6	R	3.2
66	16.7	MR	9.6
67	15.0	MR	8.6
70	9.1	R	5.2
71	44.4	S	26.4
72	14.3	MR	8.2
73	63.6	S	39.5
74	36.8	S	21.6
75	28.6	MR	16.6
76	20.0	MR	11.5
77	36.4	S	21.3
78	18.2	MR	10.5
79	47.8	S	28.6
80	6.9	R	4.0
81	27.8	MR	16.1
82	38.5	S	22.6
83	36.8	S	21.6
84	5.6	R	3.2
85	6.3	R	3.6
86	0.0	R	0.0
87	0.0	R	0.0
88	10.0	R	5.7
89	11.5	MR	6.6
90	0.0	R	0.0
92	38.9	S	22.9
93	31.3	S	18.2
94	37.5	S	22.0
95	63.6	S	39.5
96	45.5	S	27.0
97	20.0	MR	11.5
98	0.0	R	0.0
99	16.7	MR	9.6
100	8.3	R	4.8
101	43.8	S	25.9
102	24.0	MR	13.9
103	4.5	R	2.6
104	0.0	R	0.0
105	0.0	R	0.0
107	27.3	MR	15.8
108	3.8	R	2.2
109	12.5	MR	7.2
110	19.2	MR	11.1
111	11.5	MR	6.6
112	5.9	R	3.4
113	0.0	R	0.0
114	9.1	R	5.2
115	15.0	MR	8.6
116	11.1	MR	6.4
117	50.0	S	30.0
118	66.7	S	41.8
119	12.5	MR	7.2
120	72.2	S	46.2
121	22.2	MR	12.8
122	0.0	R	0.0
123	0.0	R	0.0
124	0.0	R	0.0
126	5.0	R	2.9
127	6.7	R	3.8
128	0.0	R	0.0
129	0.0	R	0.0
131	0.0	R	0.0
132	0.0	R	0.0
133	7.1	R	4.1
134	17.4	MR	10.0
136	22.2	MR	12.8
137	14.3	MR	8.2
139	16.7	MR	9.6

140	100.0	S	90.0
141	0.0	R	0.0
142	39.1	S	23.0
143	50.0	S	30.0
144	16.7	MR	9.6
145	6.7	R	3.8
146	8.7	R	5.0
147	12.5	MR	7.2
148	17.6	MR	10.2
149	30.0	S	17.5
150	29.4	MR	17.1
151	4.3	R	2.5
152	22.2	MR	12.8
153	73.7	S	47.5
154	40.0	S	23.6
155	56.5	S	34.4
156	42.3	S	25.0
157	10.5	MR	6.0
160	23.1	MR	13.3
162	26.7	MR	15.5
163	0.0	R	0.0
164	12.5	MR	7.2
166	6.7	R	3.8
167	20.0	MR	11.5
168	0.0	R	0.0
169	10.0	R	5.7
170	0.0	R	0.0
171	6.7	R	3.8
172	0.0	R	0.0
173	0.0	R	0.0
174	10.0	R	5.7
176	11.8	MR	6.8
177	16.7	MR	9.6
178	30.8	S	17.9
179	62.5	S	38.7
180	76.0	S	49.5
181	83.3	S	56.4
182	88.2	S	61.9
183	85.7	S	59.0
184	55.6	S	33.7
186	0.0	R	0.0
187	11.1	MR	6.4
188	12.5	MR	7.2
189	14.3	MR	8.2
190	0.0	R	0.0
191	11.1	MR	6.4
192	75.0	S	48.6
193	0.0	R	0.0
194	50.0	S	30.0
195	53.8	S	32.6
196	70.0	S	44.4
197	28.6	MR	16.6
198	28.6	MR	16.6
199	27.3	MR	15.8
200	0.0	R	0.0
201	0.0	R	0.0
202	0.0	R	0.0
203	0.0	R	0.0
204	5.3	R	3.0
205	0.0	R	0.0
206	8.3	R	4.8
207	15.4	MR	8.8
208	31.6	S	18.4
209	26.3	MR	15.3
210	7.7	R	4.4
211	8.3	R	4.8
212	10.0	R	5.7

213	16.7	MR	9.6
214	7.1	R	4.1
215	35.0	S	20.5
216	21.4	MR	12.4
217	40.0	S	23.6
218	33.3	S	19.5
219	7.1	R	4.1
220	33.3	S	19.5
221	18.2	MR	10.5
222	35.7	S	20.9
223	26.3	MR	15.3
224	5.6	R	3.2
225	20.0	MR	11.5
226	7.1	R	4.1
227	0.0	R	0.0
228	46.7	S	27.8
229	31.3	S	18.2
230	17.6	MR	10.2
231	5.9	R	3.4
232	55.6	S	33.7
233	44.4	S	26.4
234	11.1	MR	6.4
235	42.9	S	25.4
236	43.8	S	25.9
237	50.0	S	30.0
238	9.1	R	5.2
239	59.1	S	36.2
240	60.0	S	36.9
241	69.2	S	43.8
242	83.3	S	56.4
243	76.5	S	49.9
244	80.0	S	53.1
245	66.7	S	41.8
246	6.3	R	3.6
247	0.0	R	0.0
248	33.3	S	19.5
249	9.5	R	5.5
250	4.3	R	2.5
252	33.3	S	19.5
253	4.5	R	2.6
254	25.0	MR	14.5
255	14.3	MR	8.2
256	5.9	R	3.4
257	25.0	MR	14.5
258	10.5	MR	6.0
259	8.3	R	4.8
260	32.0	S	18.7
261	35.3	S	20.7
262	50.0	S	30.0
263	25.0	MR	14.5
264	52.6	S	31.8
265	63.2	S	39.2
266	23.8	MR	13.8
267	16.7	MR	9.6
268	36.8	S	21.6
269	27.3	MR	15.8
270	10.0	R	5.7
271	28.6	MR	16.6
272	50.0	S	30.0
273	23.5	MR	13.6
274	33.3	S	19.5
275	7.1	R	4.1
276	25.0	MR	14.5
277	40.0	S	23.6
278	26.7	MR	15.5
279	46.2	S	27.5
280	13.0	MR	7.5

281	26.7	MR	15.5
282	63.6	S	39.5
283	21.1	MR	12.2
284	13.6	MR	7.8
285	14.3	MR	8.2
286	36.4	S	21.3
287	38.9	S	22.9
288	0.0	R	0.0
289	11.8	MR	6.8
290	0.0	R	0.0
291	0.0	R	0.0
292	0.0	R	0.0
293	8.3	R	4.8
294	5.9	R	3.4
295	16.7	MR	9.6
296	12.5	MR	7.2
297	58.8	S	36.0
298	90.0	S	64.2
299	85.7	S	59.0
300	100.0	S	90.0
301	76.5	S	49.9
302	56.3	S	34.2
303	60.0	S	36.9
304	100.0	S	90.0
305	81.8	S	54.9
306	29.4	MR	17.1
307	11.8	MR	6.8
308	28.6	MR	16.6
309	25.0	MR	14.5
310	22.6	MR	13.1
311	7.7	R	4.4
312	18.5	MR	10.7
313	23.3	MR	13.5
314	0.0	R	0.0
315	26.7	MR	15.5
316	29.2	MR	17.0
317	18.2	MR	10.5
318	50.0	S	30.0
319	47.6	S	28.4
327	19.0	MR	11.0
328	21.4	MR	12.4
329	0.0	R	0.0
330	42.9	S	25.4
331	30.8	S	17.9
332	33.3	S	19.5
333	25.0	MR	14.5
341	17.2	MR	9.9
342	25.0	MR	14.5
343	30.8	S	17.9
344	26.3	MR	15.3
345	6.7	R	3.8
346	10.0	R	5.7
BSMR736	0	R	0.0
ICP 2376	60	S	36.9
Maruti	5	R	2.6

R: Resistance, MR: Moderately resistant, S: Susceptible