RESEARCH ARTICLE

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Characterization of Elite Genotypes for *Fusarium* Wilt Resistance in Pigeonpea [*Cajanus cajan* (L.) Millspaugh]

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ABSTRACT

Background: The development of *Fusarium* wilt resistant varieties is a major challenge in pigeonpea breeding and need to be addressed on priority basis. In this study, efforts had been made to characterize the elite pigeonpea genotypes for *Fusarium* wilt resistance at morphological and molecular level.

Methods: The present study was undertaken during *kharif* season of year 2018-19 at NEB, Crop Research Centre, G.B.P.U.A and T, Pantnagar. The experimental materials for present study consisted of 15 elite pigeonpea genotypes and three commercially grown popular varieties as checks. The molecular analysis was conducted during year 2019-20 and a susceptible variety BAHAR was used as check. The nine yield based indices were used for morphological analysis. The molecular analysis was carried out by using five already reported *Fusarium* wilt linked SSR markers.

Result: The results revealed that the marker ASSR 363, ASSR 366, ASSR 1, ASSR 23 and ASSR 148 were highly effective in differentiating the resistant and susceptible genotypes of pigeonpea for wilt disease. On the basis of morphological and molecular studies, it was concluded that the genotype PA 626 was the most superior genotype as it not only yielded higher than all the three checks but also shown resistance against wilt at both phenotypic and genotypic level.

Key words: *Fusarium*, Pigeonpea, SSR Markers, Stress, Wilt.

INTRODUCTION

Pigeonpea [*Cajanus cajan* (L.) Millspaugh] is a major *kharif* pulse crop of India. Pigeonpea wilt caused by a fungus *Fusarium udum* Butler severely hampers its productivity and causes yield losses upto 30 to 100% (Dhar *et al.,* 2005). The development of resistant varieties is most sustainable way to control this disease. The study of literature suggested that only few resistant sources have been identified in pigeonpea for *Fusarium* wilt (Singh *et al.,* 2016). Therefore, development of wilt resistant varieties in pigeonpea is a major challenge and needs to be addressed on priority basis. There are various yield based indices *viz.,* tolerance index, relative stress index, mean productivity, harmonic mean, yield stability index, geometric mean productivity, stress susceptibility index, stress tolerance index and yield index to characterize the genotypic response under stress conditions and to select desirable genotypes. These indices were originally developed for drought tolerance but can be effectively used to identify other abiotic and biotic stress tolerant genotypes also (Pour Aboughadareh *et al.,* 2019). Morphological screening of large number of lines is time consuming, costly and laborious process as it needs sick plots and artificial inoculation. As wilt can occurs at any stage of crop and hence rapid screening by conventional tools is not so much effective and therefore, there is need to take help from environment independent molecular markers in these types of studies. The studies conducted by various workers suggested the presence of significant marker traits association for *Fuarium* wilt of pigeonpea (Saxena *et al.* 2020; Singh *et al.* 2016). Hence, in the present study an effort had been made to integrate the different selection indices and molecular tools to characterize some elite genotypes of pigeonpea against *Fusarium* wilt.

MATERIALS AND METHODS

Plant material and field screening

The experimental materials for present study consisted of total of 18 genotypes including 15 elite pigeonpea genotypes and three commercial released popular varieties as check for grain yield (PUSA 992, UPAS 120 and PARAS). Two set of the experimental material were sown one set in wilt sick plot and other set in normal plot using randomized block

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design with three replications during the *kharif* season 2018-19 at Norman E. Borlaug, Crop Research Centre of the G.B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand), India. The wilt sick plot was created by burring the wilted stems of pigeonpea (5-8 cm long) into the soil across the field. To access the uniformity of disease incidence, one infector row of susceptible variety BAHAR was planted after every 10 rows of test genotypes. Another set was sown in normal conditions. Each genotype was sown in a single row of four meters length with row to row and plant to plant spacing of 60 and 20 cm, respectively. All recommended practices were followed to raise a normal crop.

Data collection and statistical analysis

The data on wilt incidence (%) was recorded as per the method suggested by Sharma *et al.* (2016) at three different stages *viz.,* seedling, flowering and maturity stage of crop. The cumulative incidence of all the three stages was calculated using the following formula:

$$\%\ \text{Disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

The scoring for *Fusarium* wilt reaction was done according to Nene and Kannnaiyan (1982) and Singh *et al.* (2013) as given in Table 1.

The nine yield based indices *viz.,* tolerance index (TI; Rosielle and Hamblin, 1981), relative stress index (RSI; Fischer and Wood, 1979), mean productivity (MP; Rosielle and Hamblin, 1981), harmonic mean (HM; Bidinger *et al.* 1987), geometric mean productivity (GMP; Fernandez, 1992), yield stability index (YSI; Bouslama and Schapaugh, 1984), stress susceptibility index (SSI; Fischer and Maurer, 1978), stress tolerance index (STI; Fernandez, 1992) and yield index (YI; Gavuzzi *et al.,* 1997) were estimated by using an online software programme *i*PASTIC developed by (Pour Aboughadareh *et al.,* 2019). In case of TI and SSI genotypes having minimum values were adjudged as desirable one while in other indices genotypes with maximum values were considered as desirable one.

DNA extraction and PCR

For molecular characterization, in addition to the 18 genotypes, one susceptible check BAHAR was also used. The molecular work was carried out in the Pulse Breeding laboratory of GBPUA and T, Pantnagar during 2019-20. The five *Fusarium* wilt linked molecular markers *viz.,* ASSR 1, ASSR 23, ASSR 148, ASSR 363 and ASSR 366 reported by Singh *et al.* (2013) and validated by Singh *et al.* (2016) were used in the present study. The genomic DNA was extracted by using the Cetyl tri-methyl ammonium bromide (CTAB) method of Doyle and Doyle (1987) with very slight modifications. The obtained binary data were used to compute pair wise similarity coefficients (Jaccard, 1908). The similarity matrix thus obtained was subjected to hierarchical cluster analysis using the Unweighted Pair Group Method with Arithmetic average (UPGMA) algorithm using NTSYS-pc software (Rohlf, 1988).

Table 1: Disease rating scale for *Fusarium* wilt as suggested by Nene and Kannnaiyan (1982) and Singh *et al.* (2013).

Type of reaction	% plant infected
Resistant (R)	0-20%
Moderately resistant (MR)	21-40%
Moderately susceptible (MS)	41-60%
Susceptible (S)	above 60%

RESULTS AND DISCUSSION

Per se performance of genotypes under Fusarium wilt and normal conditions

The wilt severity of different genotypes with their mean yield in stress and normal conditions are presented in Table 2. The wilt severity ranged from 10% (PA 626 and PUSA 992) to 80% (PARAS). Present findings were also supported by the earlier findings of Prasanthi *et al.* (2009). In present study out of eighteen genotypes, six genotypes *viz.,* PA 623, PA 625, PA 626, PA 627, PUSA 992 and UPAS 120 were found as resistant, seven genotypes *viz.,* PA 618, PA 619, PA 621, PA 624, PA 628, PA 629 and PA 632 was found as moderately resistant, four genotypes *viz.,* PA 620, PA 622, PA 630 and PA 631 were found as moderately susceptible and one genotype the released check variety PARAS was found as susceptible with 80% of severity. In general, the grain yield losses occurred due to disease severity as compared to normal conditions. The yield losses ranged from 10.34% in case of variety PUSA 992 to as high as 76.00% in case of PARAS. It is also evident from the Table 2 that the genotypes PA 626 (12.90%), PA 618 (16.22%) and PA 619 (16.67%) suffered less due to disease. Sharma *et al.* (2016) reported that yield loss in pigeonpea under wilt conditions was mainly due to the loss of turgidity of susceptible plants, inter-veinal chlorosis in leaves, browning of xylem vessels and extension of a purple band on stem from the base. In case of wilt conditions three genotypes *viz.,* PA 618, PA 619 and PA 626 exhibited high yield over three used checks while in case of normal conditions eight genotypes *viz.,* PA 618, PA 619, PA 620, PA 621, PA 622, PA 626, PA 628 and PA 631 exhibited higher yield over all three checks. These results indicated that three genotypes *viz.,* PA 618, PA 619 and PA 626 performed better as compared to checks under both conditions. However, out of three, one genotype PA 626 exhibited resistant reaction while the genotypes PA 618 and PA 619 exhibited moderately resistant reaction. Hence, the genotype PA 626, PA 618 and PA 619 were found as most desirable genotype as they were wilt resistant and had high yield as compared to check.

Characterization of genotypes on basis of average sum of rank methods

The results of various selection indices were shown in Table 3 and results indicated that the genotype PUSA 992 (TI=150; TI rank=1) exhibited the minimum value and first TI rank followed by genotypes PA 632, PA 626 and PA 627 each of them possess TI value of 200 and ranked jointly second. The other superior genotypes on basis of TI scores were PA 618, PA 619, PA 621 and PA 625 each having TI score equals to 300 and placed at fifth rank. As the TI index is based on the difference of yield under normal condition and yield under stress conditions hence the genotype having less deviation in yield under both conditions were found as superior by this index. The scoring pattern of SSI index was also similar to TI as in this index also the genotypes having the minimum SSI scores were considered as desirable. Genotypes PUSA 992 (SSI=0.33, SSI rank=1), PA 626 (SSI=0.41, SSI rank=2), PA 618 (SSI=0.52, SSI rank=3), PA 619 (SSI=0.53, SSI rank=4) and PA 627 (SSI=0.58, SSI rank=5) were emerged as desirable genotypes in SSI selection index. Thus, the combined results of these two indices suggested that the genotype PUSA 992, PA 626, PA 627, PA 618 and PA 619 were most superior genotypes. The results obtained from other indices like MP, HM, GMP and STI indicated that the genotype PA 618 is most superior genotype as it ranked first in all these indices followed by genotype PA 619, PA 626, PA 621 and PUSA 992 having ranked second, third, fourth and fifth rank, respectively according to these four indices. The ranks obtained by using selection indices YI indicated that PA 618, PA 619, PA 626, PUSA 992 and PA 621 were the most superior genotypes as they ranked first, second, third, fourth and fifth rank, respectively. The results of YSI and RSI indicated that genotype PUSA 992 (YSI rank=1; RSI rank=1), PA 626 (YSI rank=2; RSI rank=2), PA 618 (YSI rank=3; RSI rank=3), PA 619 (YSI rank=4; RSI rank=4) and PA 627 (YSI rank=5; RSI rank=5) were most superior genotypes. The results on basis of any single index were not much reliable and hence, an average sum of rank (ASR) based on all of these indices was used in present study to identify the superior genotypes. The results of ASR indicated that PA 618 (ASR=2.0), PA 626 (ASR=2.6), PA 619 (ASR=2.9), PUSA 992 (ASR=3.2) and PA 621 (ASR=4.9) were adjudged as most superior genotypes. These indices were earlier used by several workers in different crops (Pour Siahbidi and Pour Aboughadareh, 2013; Sardouei Nasab *et al.,* 2019).

Characterization of genotypes by using Fusarium wilt linked molecular markers

The molecular characterization of 19 pigeonpea genotypes including the susceptible check BAHAR revealed that the markers ASSR 363 was the most informative marker on the basis of highest PIC value (0.66) (Table 4). Effectiveness of markers identified in present study in differentiating resistant and susceptible genotypes were supported by earlier findings of Singh *et al.* (2016). The result of present study further confirmed that these five markers could be utilized in the marker assisted breeding programme for identifying *Fusarium* wilt resistance in pigeonpea. In present study the used markers differentiated the population into three different clusters (Table 5; Fig 1). The cluster-II was the largest as it contains 10 genotypes followed by cluster-III containing five genotypes and cluster-I containing four genotypes (Table 5). A close perusal of results depicted in Table 5 indicated that largest number of resistant genotypes were present in Cluster-II followed by cluster-III. The cluster-I did not contain any resistant genotypes and this cluster contains the

Table 2: The wilt severity of genotypes with their mean yield in stress and normal conditions.

Genotypes	Wilt severity (%)	Disease response	M_{YS} [Mean yield in stress condition (kg/ha)]	M_{YN} [Mean yield in normal condition (kg/ha)]	Yield loss per cent [$(M_{YN} - M_{YS} / M_{YN}) \times 100$]
PA 618	30%	MR	1550	1850	16.22
PA 619	30%	MR	1500	1800	16.67
PA 620	50%	MS	900	1650	45.45
PA 621	30%	MR	1250	1550	19.35
PA 622	50%	MS	850	1750	51.43
PA 623	20%	R	800	1150	30.43
PA 624	30%	MR	900	1250	28.00
PA 625	20%	R	800	1100	27.27
PA 626	10%	R	1350	1550	12.90
PA 627	20%	R	900	1100	18.18
PA 628	30%	MR	1100	1550	29.03
PA 629	40%	MR	550	950	42.11
PA 630	50%	MS	750	1500	50.00
PA 631	50%	MS	800	1550	48.39
PA 632	40%	MR	550	750	26.67
PUSA 992	10%	R	1300	1450	10.34
UPAS 120	20%	R	1150	1500	23.33
Paras	80%	S	300	1250	76.00

Where, R= Refers to resistant, MR= Refers to moderately resistant, MS= Moderately susceptible, S= Refers to susceptible.

Table 3: Evaluation of genotypes on basis of nine stress indices.

Genotypes	Ys	Ys Rank	TI	TI Rank	MP	MP Rank	HM	HM Rank	GMP	GMP Rank	SSI	SSI Rank	STI	STI Rank	YI	YI Rank	YSI	YSI Rank	RSI	RSI Rank	ASR
PA 618	1550	1	300	5	1700	1	1686.77	1	1693.37	1	0.52	3	1.46	1	1.61	1	0.84	3	1.22	3	2
PA 619	1500	2	300	5	1650	2	1636.36	2	1643.17	2	0.53	4	1.37	2	1.56	2	0.83	4	1.22	4	2.9
PA 620	900	8	750	14	1275	9	1164.71	8	1218.61	9	1.44	14	0.75	9	0.94	8	0.55	14	0.80	14	10.7
PA 621	1250	5	300	5	1400	4	1383.93	4	1391.94	4	0.61	6	0.98	4	1.30	5	0.81	6	1.18	6	4.9
PA 622	850	11	900	17	1300	8	1144.23	9	1219.63	8	1.63	17	0.76	8	0.88	11	0.49	17	0.71	17	12.3
PA 623	800	12	350	9	975	14	943.59	14	959.17	14	0.97	12	0.47	14	0.83	12	0.70	12	1.02	12	12.5
PA 624	900	8	350	9	1075	12	1046.51	11	1060.66	11	0.89	10	0.57	11	0.94	8	0.72	10	1.05	10	10
PA 625	800	12	300	5	950	15	926.32	15	938.08	15	0.87	9	0.45	15	0.83	12	0.73	9	1.06	9	11.6
PA 626	1350	3	200	2	1450	3	1443.10	3	1446.55	3	0.41	2	1.06	3	1.40	3	0.87	2	1.27	2	2.6
PA 627	900	8	200	2	1000	13	990.00	13	994.99	13	0.58	5	0.50	13	0.94	8	0.82	5	1.19	5	8.5
PA 628	1100	7	450	13	1325	6	1286.79	7	1305.76	7	0.92	11	0.87	7	1.14	7	0.71	11	1.04	11	8.7
PA 629	550	16	400	12	750	17	696.67	16	722.84	16	1.34	13	0.27	16	0.57	16	0.58	13	0.84	13	14.8
PA 630	750	15	750	14	1125	11	1000.00	12	1060.66	11	1.59	16	0.57	11	0.78	15	0.50	16	0.73	16	13.7
PA 631	800	12	750	14	1175	10	1055.32	10	1113.55	10	1.54	15	0.63	10	0.83	12	0.52	15	0.75	15	12.3
PA 632	550	16	200	2	650	18	634.62	17	642.26	17	0.85	8	0.21	17	0.57	16	0.73	8	1.07	8	12.7
PUSA 992	1300	4	150	1	1375	5	1370.91	5	1372.95	5	0.33	1	0.96	5	1.35	4	0.90	1	1.31	1	3.2
UPAS 120	1150	6	350	9	1325	6	1301.89	6	1313.39	6	0.74	7	0.88	6	1.20	6	0.77	7	1.12	7	6.6
PARAS	300	18	950	18	775	16	483.87	18	612.37	18	2.41	18	0.19	18	0.31	18	0.24	18	0.35	18	17.8

Where, Ys= Yield under stress; TI = Tolerance index, MP= Mean productivity; HM= Harmonic mean; GMP= Geometric mean productivity; SSI= Stress susceptibility index; STI= Stress tolerance index; YI= Yield index; YSI= Yield stability index; RSI= Relative stress index; ASR= Average sum of ranks.

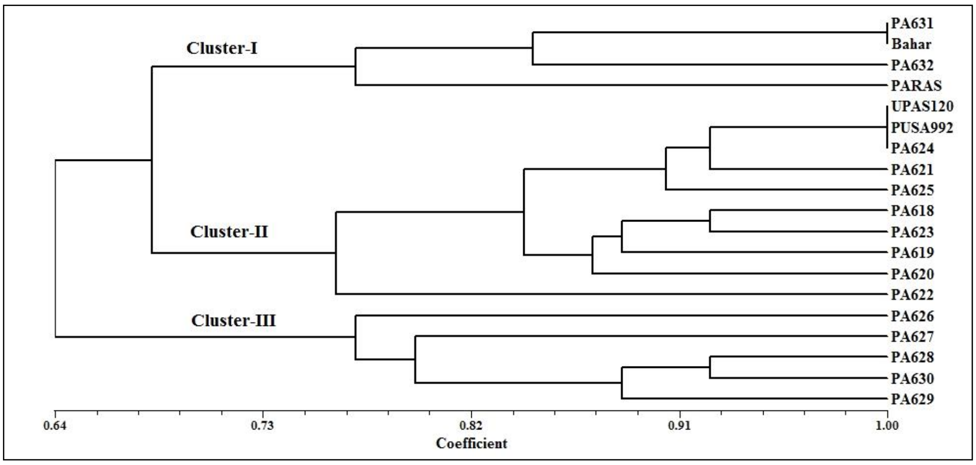

Fig 1: Dendrogram obtained by using *Fusraium* wilt linked markers in pigeonpea.

Table 4: Polymorphism features of used SSR markers.

Primer	Allele size range (bp)	PICValue
ASSR 1	100-390	0.45
ASSR 23	100-120	0.42
ASSR 148	100-150	0.42
ASSR 363	100-150	0.66
ASSR 366	150-180	0.63

Table 5: The clusters obtained by using *Fusarium* wilt linked markers.

Clusters	Number of resistant (R) genotypes	Genotypes
Cluster-I	0	PA 631, BAHAR, PA 632 and PARAS
Cluster-II	4	UPAS 120, PUSA 992, PA 624, PA 621, PA 625, PA 618, PA 623, PA 619, PA 620 and PA 622
Cluster-III	2	PA 626, PA 627, PA 629, PA 630 and PA 628

susceptible check BAHAR along with other susceptible genotypes like PARAS and PA 631 besides a moderately resistant genotype PA 632. Present findings strongly indicated that used markers were highly effective in differentiating the resistant and susceptible genotypes. However, in case of moderately resistant and moderately susceptible genotypes these markers are partial effective. Similar to present finding, Singh *et al.* (2013) also reported that wilt resistance and susceptible genotypes can be differentiated into different clusters by using the molecular markers. Swami *et al.* (2015) compared the efficiency of RAPD, ISSR and SSR markers in differentiating the wilt resistant and susceptible genotypes in pigeonpea and observed that SSR and ISSR markers were effective in differentiating the genotypes in resistant and susceptible groups. In the present study, the results of cluster analysis indicated that the cluster-II and cluster III were the most desirable clusters as these clusters contain wilt resistant genotypes. The most desirable genotypes having wilt resistance along with high yield in cluster II were identified as PUSA 992 (R; yield= 1300) and UPAS 120 (R; yield= 1150) while the most desirable genotype in cluster III was PA 626 (R; yield= 1350). The wilt resistant genotypes included in cluster II and III can either be directly released as a new variety after proper yield evaluation and/or can be used as donor parents for incorporating wilt resistance through hybridization programme.

CONCLUSION

The results of average sum of rank (ASR) based on nine indices indicated that PA 618, PA 626, PA 619, PUSA 992 and PA 621 were most superior genotypes. The results of molecular analysis revealed that marker ASSR 363, ASSR 366, ASSR 1, ASSR 23 and ASSR 148 could be utilized in the marker assisted breeding programme for incorporating *Fusarium* wilt resistance in pigeonpea. On the basis of morphological as well as molecular studies, it can be concluded that the genotype PA 626 turned out not only as top yielder but also shown resistance against wilt at both phenotypic and genotypic level.

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