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Screening and Validation of Drought Tolerance and *Fusarium* Wilt Resistance in Advance Breeding Lines of Chickpea (*Cicer arietinum* L.)

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ABSTRACT

Background: Approximately 90% of the world's chickpea is grown under rainfed conditions where terminal drought is one of the major constraints limiting productivity. The need of short-duration, *Fusarium* wilt tolerant cultivars/elite lines and able to escape drought due to early maturity were required.

Methods: The present investigation was carried out using 54 genotypes, generated from six diverse crosses, along with ten checks (resistant/tolerance, susceptible) were screened against drought and *Fusarium* wilt at Zonal Agricultural Research Station, Kalaburagi, Karnataka (Latitude: 17.36 and Longitude: 76.82) during crop season 2018-19.

Result: The results revealed that higher PCV, GCV, heritability, per cent genetic advance were exhibited by number of pods per plant and seed yield per plot, whereas lower PCV, GCV recorded for days to 50% flowering and days to maturity in both normal and late sown conditions. The advanced breeding lines *viz.*, KCD-8, KCD-24, KCD-28, KCD-32, KCD-37 and KCD-53 were identified as drought tolerant lines based on drought tolerant indices (*viz.*, MP, YSI, DTE and DSI). The lines KCD-48 and KCD-32 were identified as *Fusarium* wilt resistance with lowest PDI of 1.47 and 2.46 respectively, as they were screened in wilt sick plot and further these were validated and confirmed the resistant alleles using two unpublished SNP markers (FW2_30366110 and FW2_30365816). The advanced breeding lines KCD-32 and KCD-37 were identified as drought tolerant and *Fusarium* wilt resistant.

Key words: ABLs, Chickpea, Drought, *Fusarium* wilt, Markers.

INTRODUCTION

Chickpea (*Cicer arietinum* L.), popularly known as Gram, Bengal gram, Egyptian pea, Chana, or Garbanzo bean, is one of the first grain legumes to have been domesticated by humans in the old world (Van der Maesen, 1984). Being a cool season crop, chickpea is often grown over a wide range of environments, from subtropical to temperate. In India, chickpea is cultivated over an area of about 9.67 million hectares with the production of 10.09 million tonnes with a productivity of 1043 kg ha^{-1}. In Karnataka, it is cultivated in a total area of 1265 thousand hectares with production of 783 thousand tonnes having productivity of 619 kg ha^{-1} (Directorate of Economics and Statistics, 2018-19) and Karnataka is one of the major chickpea producing state in the country.

Lower productivity of chickpea is ascribable to the susceptibility of cultivars to several biotic and abiotic stresses. Drought is one of the most important constraints, among abiotic stresses, limiting yield potential in both cereal and legume crops. It was well documented that drought stress during pod filling can lead to pod abortion thus reducing the number of seeds per plant (Fang *et al*., 2010; Pang *et al*., 2017). Approximately 90% of the chickpea is grown under rainfed conditions where terminal drought is one of the major constraints limiting productivity. *Fusarium* wilt is one of the major abiotic stresses and it is soil borne pathogen affecting chickpea globally and epidemics can be devastating and cause losses up to 100% in highly infected fields under favourable conditions to pathogen (Jendoubi *et al*., 2017). Sometimes under favourable conditions, there is a total failure of crop and eventually yield (Navas-Cortés *et al*., 2000). Combining drought tolerance and *Fusarium* wilt resistance is the need of the hour because both are major constraints in chickpea production.

In the last decade, the publications on development and application of molecular markers in plant breeding have increased exponentially (Xu and Jonathan, 2008). Published

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markers need to be validated in the range of population representatives to be routinely screened. In this context, validation of markers and their utilization in marker assisted selection (MAS) was felt very important. Keeping above in view this study has undertaken detailed phenotypic and molecular characterization of chickpea advance breeding lines for drought and *Fusarium* wilt.

MATERIALS AND METHODS

The experimental material comprised of 54 advanced breeding lines (ABLs), generated and maintained from six diverse parental crosses, with ten check varieties *viz*., MABC-WR-SA-1, WR-315, JG-62, MLT-66-266, ICCV-4958, ICCV-10, MLT-411-111, JG-11, A-1, GBM-2. These ABLs were obtained through pedigree method and selection was carried out in wilt sick plot at ZARS, Kalaburagi from 2017 to 2019. The experiment was laid out in Lattice Design (8 × 8) with two replications. Each genotype was sown in 2 rows of 4 meter length with a spacing of 30 cm and 10 cm between rows and plants respectively. Sowing was undertaken by hand dibbling method and approximately 40 seeds were sown per genotype. Normal season sowing was done on 12th October, 2019 and late sowing was done on 21st November, 2019 for drought screening (Plate 1) at Zonal Agricultural Research Station, Kalaburagi during 2018-19. In order to identify and ascertain the genetic variability among the genotypes and to confirm the presence of environmental effect on various characteristics of genotypes, different genetic parameters were estimated by different methods. Both genotypic and phenotypic coefficients were computed for each character as per the method suggested by Burton and Davane (1953), GCV and PCV values were categorized as low, moderate and high values as suggested by Sivasubramanian and Menon (1973), Heritability in broad sense was computed as suggested by Hansen *et al.* (1956) and expressed as percentage. The heritability percentage was low, moderate and high as given by Robinson *et al.* (1949), genetic advance was estimated by using the formula as suggested by Johnson *et al.* (1955) and Genetic advance as per cent mean was categorized as low, moderate and high as given by Johnson *et al.* (1955). The response of genotypes to moisture stress was assessed by Mean productivity (MP) by Rosielle and Hamblin, 1981, Yield stability index (YSI) by Bouslama and Schapaugh, 1984, Drought tolerance efficiency (DTE) by Fisher and Wood, 1981and Drought susceptibility index (DSI) by Fisher and Maurer, 1978. Experimental layout for screening *Fusarium* wilt was laid out on National Wilt Sick Plot maintained at Zonal Agricultural Research Station, Kalaburagi [Latitude (N) 17° 35′ and Longitude (E) 76° 81′] during 2018-19. All the genotypes were sown in single row along with wilt susceptible (JG-62) and resistant check varieties (WR-315) during the *Rabi* 2019 season (Plate 2). A row length of 4 meters each was maintained with a spacing of 30 cm and 10 cm between the rows and plants respectively. The observations on per cent disease incidence was recorded at 30, 60, 90 days after sowing by counting the number of diseased and dead plants (due to *Fusarium* wilt) among the total number of plants present per genotype and per cent disease incidence was estimated. Two allele specific SNP makers were used to study



Plate 1: Drought tolerance reaction in advanced breeding lines of chickpea.



Plate 2: *Fusarium* wilt disease reaction in advanced breeding lines of chickpea.

the association of allele with *Fusarium* wilt (FW) for validation. Among these two markers, FW2_30366110 was linked to *Fusarium* wilt resistance and FW2_30365816 was linked to susceptibility (Veenashri *et al.* (2020). Marker validation work was carried out at Centre of excellence in Genomics (CEG) lab ICRISAT, Hyderabad during crop season 2018-19.

RESULTS AND DISCUSSION

Genetic variability studies

The genetic variability parameters *viz.,* mean, range, genotypic co-efficient of variation (GCV), phenotypic co-efficient of variation (PCV), heritability in broad sense (h^2bs) and expected genetic advance over per cent of mean (GAM) of all character in both conditions are presented in Table 1 and the comparison of GCV and PCV between normal and late sown plot are depicted in Fig 1 and Fig 2. The results revealed that higher PCV, GCV, heritability, percent genetic advance were exhibited by number of pods per plant and seed yield per plot, whereas lower PCV, GCV for days to 50% flowering and days to maturity in both normal and late sown conditions. Similar findings were recorded by Banik *et al.* (2018) and Mayuriben *et al.* (2019).

Identification of drought tolerant genotypes

There are several methods to evaluate genetic differences

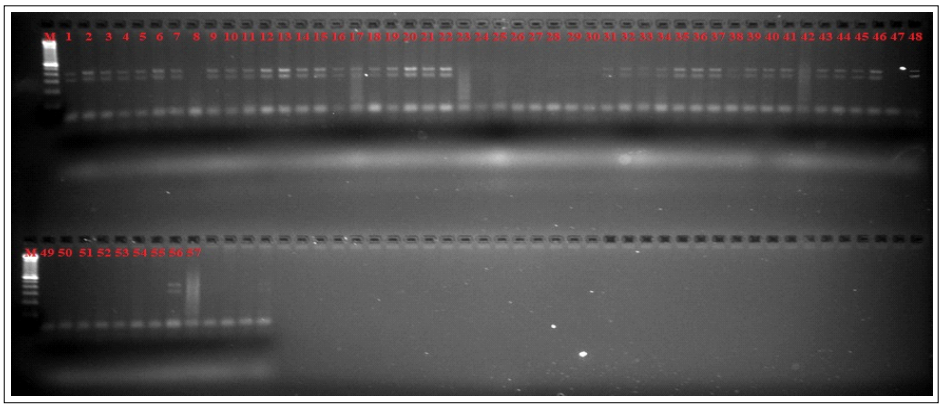

Plate 2a: Representative gel image of FW2_30366110 validation against *Fusarium* wilt.

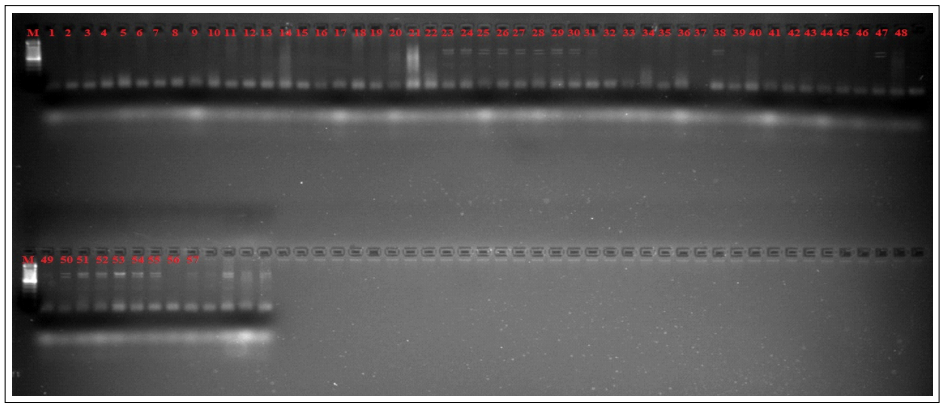

Plate 2b: Representative gel image of FW2_30365816validation against *Fusarium* wilt.

Legends:

M	Marker	10	KCD-10	20	KCD-20	30	KCD-30	40	KCD-40	49	KCD-49
1	KCD-1	11	KCD-11	21	KCD-21	31	KCD-31	41	KCD-41	50	KCD-50
2	KCD-2	12	KCD-12	22	KCD-22	32	KCD-32	42	KCD-42	51	KCD-51
3	KCD-3	13	KCD-13	23	KCD-23	33	KCD-33	43	KCD-43	52	KCD-52
4	KCD-4	14	KCD-14	24	KCD-24	34	KCD-34	44	KCD-44	53	KCD-53
5	KCD-5	15	KCD-15	25	KCD-25	35	KCD-35	45	KCD-45	54	KCD-54
6	KCD-6	16	KCD-16	26	KCD-26	36	KCD-36	46	KCD-46	55	JG-62
7	KCD-7	17	KCD-17	27	KCD-27	37	KCD-37	47	KCD-47	56	WR-315
8	KCD-8	18	KCD-18	28	KCD-28	38	KCD-38	48	KCD-48	57	SA-1
9	KCD-9	19	KCD-19	29	KCD-29	39	KCD-39	M	Marker		

for drought amongst the genotypes. It was therefore, planned to find precise field techniques to detect genotypic differences for drought tolerance and also to soar up the higher yield production in the aftermath of the drought. Drought tolerant indices in genotypes with respect to yield (kg ha^{-1}) are given in Table 2. The Mean productivity (MP) values of the genotypes were ranged from 1955 to 766. The higher mean productivity were observed in KCD-24, KCD-48, KCD-53, KCD-41 and KCD-2 indicating that these genotypes are drought tolerant and maybe suitable for both stressed and non-stressed conditions. Similar findings were recorded by Sabaghnia and Janmohammadi (2014).

Yield stability index (YSI) is used to identify the stability of genotypes in terms of yield. The YSI values of the genotypes were ranged from 1.67 to 0.41. The highest values was observed in KCD-28, KCD-32, KCD-8, KCD-24 and KCD-37 indicating that these genotypes are stable

Table 1: Genetic variability parameters for different traits in chickpea under normal and late sown conditions.

Character		Mean	Range		Coefficient variation		h^2 bs(%)	GA(5%)	GAM (5%)
			Minimum	Maximum	GCV (%)	PCV(%)			
Days to 50% flowering	NS	45.35	36.00	50.50	6.01	7.33	67	4.60	10.14
	LS	44.93	38	49	4.73	4.80	96	4.31	9.59
Days to maturity	NS	83.96	78.50	89.00	4.18	4.31	93	7.00	8.33
	LS	82.88	72	87	3.44	3.46	99	5.85	7.06
Plant height (cm)	NS	42.19	29.67	53.50	12.91	15.75	67	9.19	21.79
	LS	36.75	28.17	46.67	10.61	14.60	52	5.83	15.88
No. of primary branches/plant	NS	2.99	2.00	4.33	9.90	19.78	25	0.30	10.22
	LS	3.03	1.83	4.17	16.17	24.53	43	0.66	21.96
No. of secondary branches/plant	NS	4.13	1.33	8.00	20.04	38.39	27	0.89	21.55
	LS	4.15	2.13	6.50	13.51	27.94	23	0.55	13.46
No. pods/plant	NS	27.71	16.83	52.50	21.38	25.00	73	10.43	37.65
	LS	18.31	10.50	34.67	24.09	27.19	78	8.05	43.97
No. seeds/pod	NS	1.17	1.00	1.55	7.55	12.49	36	0.11	9.40
	LS	1.04	1.00	1.22	5.41	6.16	77	0.10	9.80
100-seed weight	NS	18.94	14.19	23.60	7.15	11.53	38	1.73	9.14
	LS	18.41	13.54	23.52	9.48	11.72	65	2.91	15.80
Seedling vigour	NS	2002.09	1450.00	2893.75	14.44	15.83	83	543.37	27.14
	LS	1367.15	458.38	2325	36.79	36.93	99	1032.56	75.52
Seed yield/plot (kg ha^{-1})	NS	1519.36	813	2618	26.65	28.00	90	794.39	52.28
	LS	1243.01	500	2114	29.52	30.98	90	720.29	57.94

NS = Normal sown; LS = Late sown; * = Mechanical harvesting type.

Where, h^{2}bs= Heritability in broad sense; GAM= Genetic advance as per cent mean; GCV= Genotypic coefficient of variability; GA= Genetic advance; PCV= Phenotypic coefficient of variability.

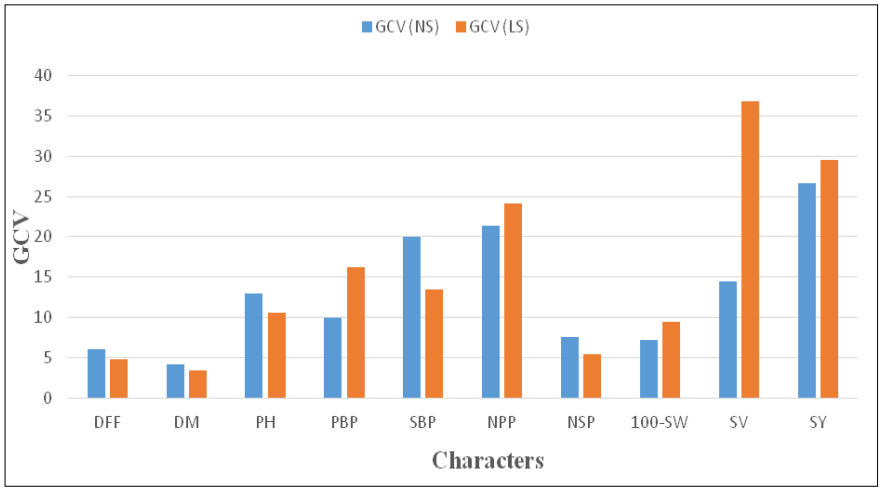

Fig 1: Comparison of genotypic coefficient of variation (GCV) between normal and late sown conditions.

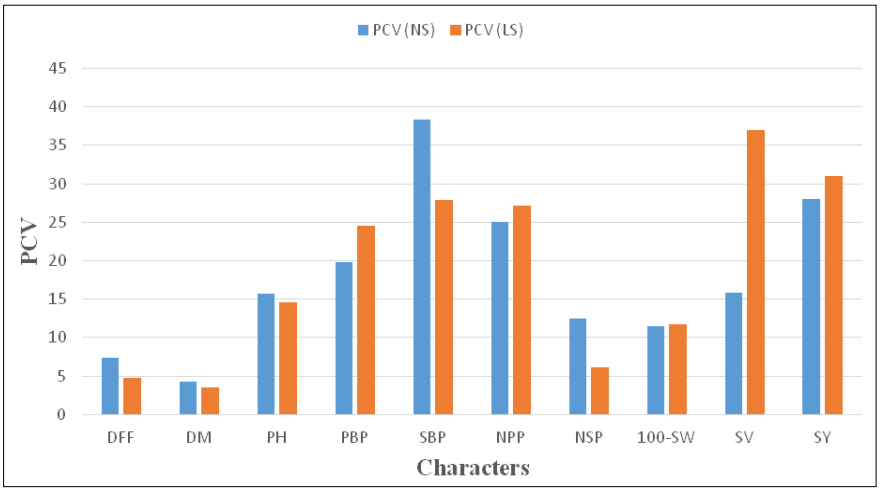

Fig 2: Comparison of phenotypic coefficient of variation (PCV) between normal and late sown conditions.

performer in terms of yield and identified as drought tolerant genotypes which maybe suitable for both stressed and non-stressed conditions. The results are in accordance with the findings of earlier workers *viz.,* Sabaghnia and Janmohammadi (2014) and Derya *et al.* (2017). Drought tolerance efficiency (DTE) value of the genotypes was ranged from 166.70 to 41.44. The highest value of DTE was recorded in KCD-28, KCD-32, KCD-8, KCD-24 and KCD-37 compared to drought check ICCV-4958 indicating that these genotypes are drought tolerant and maybe desirable for both irrigated and rainfed conditions. Similar findings were recorded by Hussain *et al.* (2015) and Erdemci (2018). Drought susceptibility index (DSI) value of the genotypes was ranged from 3.25 to 0.05. The genotypes with low DSI values are drought tolerant because they have lesser reduction in grain yield under stress condition. The lowest DSI values are observed in KCD-7, KCD-29, KCD-14, KCD-52 and KCD-53 indicating that these genotypes are drought tolerant. Similar findings were recorded by Ulemale *et al.* (2013).

Field screening of genotypes against *Fusarium* wilt

Fusarium wilt disease is one of the most destructive diseases in chickpea, which is caused by *Fusarium oxysporum f. sp. ciceri.* Since it is a soil borne fungus, it can persist in a soil for a longer period of time in the form of clamydospores. Early wilting causes huge loss than wilting at later growth stages and they produce seeds which are lighter, dull and rough compared to seeds of the healthy. Present study, 17 genotypes out of 54 (31.81%) showed resistance reaction to *Fusarium* wilt (FW). The per cent disease incidence (PDI) ranged from 1.47 (KCD-48) to 9.67 (KCD-16) and the score for resistant check WR-315 and MABC-WR-SA-1 was 6.66 and 6.81% respectively. Moderately resistant reaction for *Fusarium* wilt was observed in 32 genotypes (59.25%) with PDI ranging from 10.12 (KCD-50) to 19.44% (KCD-19 and KCD-31). There were 5 out of 54 genotypes (9.25%) showed susceptible reaction to *Fusarium* wilt with PDI ranged from

Table 2: Drought tolerant indices in genotypes with respect to yield (kg ha^{-1}).

Entry	Mean productivity (kg)	Yield stability index	Drought tolerant efficiency	Drought susceptibility index
KCD-1	1251	0.51	50.87	2.73
KCD-2	1833	0.81	81.24	1.04
KCD-3	1577	0.93	93.12	0.38
KCD-4	1774	0.56	55.56	2.47
KCD-5	1514	0.87	86.90	0.73
KCD-6	1097	0.93	93.07	0.38
KCD-7	1359	1.01	100.92	0.05
KCD-8	1331	1.20	119.74	0.92
KCD-9	880	0.56	56.29	2.43
KCD-10	838	0.49	48.75	2.85
KCD-11	1034	1.07	106.61	0.34
KCD-12	1259	0.79	79.27	1.15
KCD-13	1160	0.89	88.70	0.63
KCD-14	1411	0.98	98.40	0.09
KCD-15	1164	0.84	84.40	0.87
KCD-16	1106	0.84	83.77	0.90
KCD-17	1102	0.83	83.09	0.94
KCD-18	1029	0.93	93.10	0.38
KCD-19	1035	0.86	86.40	0.76
KCD-20	1399	0.71	70.55	1.64
KCD-21	982	0.83	83.42	0.92
KCD-22	1065	0.68	68.13	1.77
KCD-23	1794	1.13	112.92	0.64
KCD-24	1955	1.18	117.70	0.84
KCD-25	1794	0.57	56.80	2.40
KCD-26	1447	1.05	105.32	0.28
KCD-27	1458	0.94	94.18	0.32
KCD-28	1236	1.67	166.70	2.22
KCD-29	851	1.01	101.40	0.08

Table 2: Continue.....

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KCD-30	1741	0.83	83.32	0.93
KCD-31	766	0.74	74.09	1.44
KCD-32	1262	1.20	119.85	0.92
KCD-33	1704	0.72	71.79	1.57
KCD-34	1715	1.07	106.53	0.34
KCD-35	1511	0.68	67.51	1.80
KCD-36	1233	0.75	75.15	1.38
KCD-37	1572	1.17	116.85	0.80
KCD-38	951	0.69	68.83	1.73
KCD-39	1127	0.92	91.74	0.46
KCD-40	884	0.47	46.63	2.97
KCD-41	1851	0.41	41.44	3.25
KCD-42	1381	0.93	92.73	0.40
KCD-43	948	1.06	106.00	0.31
KCD-44	905	0.82	82.28	0.98
KCD-45	1073	0.62	62.17	2.10
KCD-46	849	1.09	108.93	0.46
KCD-47	1593	0.95	95.46	0.25
KCD-48	1899	1.17	116.82	0.80
KCD-49	1547	0.63	62.86	2.06
KCD-50	1602	0.84	83.88	0.90
KCD-51	1680	1.04	104.02	0.21
KCD-52	1826	0.98	97.63	0.13
KCD-53	1859	1.03	103.49	0.19
KCD-54	1721	0.78	78.28	1.21
MABC-WR-SA-1 (C)	1708	0.75	75.03	1.39
WR-315 (C)	1641	0.65	65.34	1.93
JG-62 (C)	963	0.35	35.06	3.61
MABC-66-466 (C)	2025	0.62	62.44	2.09
ICCV-4958 (C)	1760	0.56	56.10	2.44
ICCV-10 (C)	1088	0.41	41.42	3.25
MLT-411-111 (C)	1567	0.89	89.12	0.60
JG-11 (C)	1704	0.80	79.69	1.13
A-1 (C)	1542	0.66	66.50	1.86
GBM-2 (C)	1462	0.87	87.40	0.70

20.31 (KCD-11) to 51.42% (KCD-29) and the PDI for susceptible check JG-62 was 100%. The details on genotypes showing resistant reaction to *Fusarium* wilt are presented in Table 3. Similar study was done by Kumar *et al*. (2019) they evaluated 55 genotypes in sick plot and identified one resistant and 12 moderately resistance genotypes.

Validation of markers linked to *Fusarium* wilt

Screening genotypes in a wilt sick plot coupled with validation by molecular markers has indicated to increase efficiency of selection and breeding for *Fusarium* wilt resistance in chickpea. In the present study, two allele specific SNP markers *viz.,* FW2_30366110 and FW2_30365816 were used to validate and confirmation of the genotypes for resistance to *Fusarium* wilt. Among these two markers, FW2_30366110 was found linked to *FOC* 4 locus of *Fusarium* wilt resistance and FW2_30365816 was linked to susceptibility.

ABLs chosen for present investigation were of different genetic background which were phenotypically screened earlier and characterized for wilt reaction have been used for confirmation of resistance using two SNP markers.

Table 3: Resistant lines identified for *Fusarium* wilt among 54 chickpea genotypes under field condition.

Sl. no.	Entry name	Per cent disease incidence (%)	Reaction
1	KCD-48	1.47	R
2	KCD-54	2.17	R
3	KCD-32	2.46	R
4	KCD-44	3.12	R
5	KCD-5	3.44	R
6	KCD-3	3.63	R
7	KCD-37	4.54	R
8	KCD-14	4.68	R
9	KCD-34	4.76	R
10	KCD-20	6.25	R
11	KCD-47	6.45	R
12	KCD-10	6.66	R
13	KCD-17	6.66	R
14	KCD-15	7.31	R
15	KCD-4	7.84	R
16	KCD-21	8.88	R
17	KCD-16	9.67	R
Resistant check	WR-315	6.66	R
Susceptible check	JG-62	100	HS

Table 4: Confirmation of *Fusarium* wilt resistance ABLs using SNP markers.

Genotype	Phenotypic wilt reaction	FW2_30366110	FW2_30365816
KCD-3	R	√	×
KCD-4	R	√	×
KCD-5	R	√	×
KCD-10	R	√	×
KCD-14	R	√	×
KCD-15	R	√	×
KCD-16	R	√	×
KCD-17	R	√	×
KCD-20	R	√	×
KCD-21	R	√	×
KCD-32	R	√	×
KCD-34	R	√	×
KCD-37	R	√	×
KCD-44	R	√	×
KCD-47	R	×	√
KCD-48	R	√	×
KCD-54	R	×	√
WR-315 (RC)	R	√	×
JG-62 (SC)	S	×	√

Where, √ = Presence of respective allele; × = Absence of respective allele.

The details on the confirmation of *Fusarium* wilt resistance lines using SNP markers are presented in Table 4, Plate 2a and Plate 2b. Out of 17 resistant lines confirmed with sick plot screening 15 showed the presence of resistant allele by specific SNP marker FW2_30366110. Similar findings were reported by Veenashri *et al.* (2020) who validated 22 advanced breeding lines of cross JG-11 x WR-315 and they found three lines were validated for the presence of wilt resistant by allele specific SNP marker FW2_30366110.

CONCLUSION

The advanced breeding lines *viz.,* KCD-8, KCD-24, KCD-28, KCD-32, KCD-37 and KCD-53 were identified as drought tolerant lines based on drought tolerant indices (*viz*., MP, YSI, DTE and DSI). The lines KCD-32 and KCD-37 were identified as *Fusarium* wilt resistance with PDI of 2.46 and 4.45 respectively, as they were screened in wilt sick plot and further these were confirmed using two SNP markers (FW2_30366110 and FW2_30365816). The advanced breeding lines KCD-32 and KCD-37 were identified as drought tolerant and *Fusarium* wilt resistant. These lines further can be used as parent in hybridization programme or directly released as a variety.

Conflict of interest

All authors declared that there is no conflict of interest.

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