

ROOT ANATOMICAL PARAMETERS FOR IDENTIFICATION OF FUSARIUM WILT RESISTANT AND SUSCEPTIBLE LINES OF CHICKPEA

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ABSTRACT

Six known *Fusarium* wilt resistant and six susceptible lines of chickpea were taken for the study of root morphological and vascular anatomical features of fifteen days old seedlings grown in plastic pot without inoculation. Resistant lines had comparatively fewer numbers of lateral roots as compared to susceptible lines. Microscopic observation transverse section of tap root has shown some correlation between resistant traits and vascular anatomy. Resistant lines showed a tendency of higher protoxylem and metaxylem number and total xylem vessels cross-section area. However, some chickpea lines possessing less number of protoxylem and metaxylem showed susceptible response. The root anatomical parameters could differentiate the *Fusarium* wilt resistant and susceptible lines of chickpea with the help of scattered plot diagram.

KEYWORDS: *Fusarium* wilt, chickpea, vascular anatomy, root morphology

INTRODUCTION

Chickpea (*Cicer arietinum*) is one of the most important food legumes grown worldwide, especially in the dry areas of the Indian subcontinent (Saxena 1990) and the second most important pulse crop of the world. It is a deep rooted crop belonging to the family Fabaceae. The cultivated chickpea (*Cicer arietinum* L.) was one of the first grain legumes domesticated from ancient time. Chickpea is originated in an area of South Eastern Turkey and adjoining Syria. It is now widely cultivated in Europe, Persia, Egypt, India, Central and Southern America, Australia and parts of Africa.

Chickpea is high protein legume grown in India. It is grown on about 9.21 million ha with production of 8.88 million ton with productivity of 995 kg/ha (Singh and Sewak, 2013). India is the world's largest chickpea growing country having area of 6.5 million hectares with the production of 5.77 million tonnes with an average yield of 888 kg/ha (Deshmukh, 2005) and thus contributes about 63% to the global production of chickpea (Rathod and Vakharia, 2011). In Maharashtra, chickpea is grown on 13.24 lakh ha with production of 12.14 lakh tonnes and productivity of 917 kg/ha. Chickpea production has gone up 3.65 to 5.63 metric tons from 1950-51 and 2004-05 registering growth of 0.58 per cent per annually (Anonymous, 2008).

The major constraints limiting chickpea production is difficulty in managing diseases caused by soil borne pathogens viz., *Fusarium* wilt, *Verticillium* wilt, dry root rot, wet root rot, black root rot, seed rot, collar rot and foot rot (Nene and Reddy 1987).

The fungus *F. oxysporum* f. sp. *ciceri* (Foc), is both seed and soil borne and persists in soil in the absence of chickpea for more than five years (Kraft et al., 1994). Booth, (1971) described *F. oxysporum* as a cosmopolitan soil-borne filamentous fungus. It is an anamorphic species that includes numerous plant pathogenic strains causing wilt diseases of a broad range of agricultural and ornamental host plant species (Appel and Gordon, 1996). The high pathogenic variability in populations of *F. oxysporum* f. sp. *ciceri* presents problems for sustainability of resistant cultivars. Two pathotypes and eight races of the pathogen have been identified. The yield loss due to this disease also depends upon the stage at which the plants wilt and it can approach over 50 % and even up to 100 % when wilt occurs at the pre-pod stage. (Okiror, 2002). No satisfactory control is found to protect the crop from wilt. Use of resistance and tolerance trait forms a vital strategy to manage the wilt disease. Therefore, breeding for resistant varieties seems to be the only option. Molecular markers for identification of wilt resistant line of chickpea have also been developed (Reddy et al., 2009; Kotresh et al., 2006).

Chickpea wilt is predominantly a soil-borne disease. Entry into the host occurring through lateral roots and partial wilt is the indication of pathogen's progress through particular vascular channel only. Resistance against chickpea wilt has been ascribed to slow growth in xylem vessels and vascular bundle of resistant cultivars (Pandey et al., 1997; Chaudhary and Kumar, 2000). Although several resistant or tolerant cultivars or lines are known, however, no standard evaluation technique has been developed so far. Evaluation of resistance is done in conventional sick-plot methods. The evaluations of germplasm and management trials are becoming difficult and questionable. Therefore, the present studies were undertaken to link wilt resistance to plant morphological and anatomical traits for indirect selection of genotypes and characterization of anatomical markers for resistant and susceptible cultivars may provide a basis for selection of wilt resistance in breeding programme.

MATERIALS AND METHODS

Collection of genotypes of chickpea

Seeds of six resistant lines viz., WR 315, Digvijay, Vishal, Virat, Vihar and Vijay and seeds of six susceptible lines viz., JG 62, Vikas, Vishwas, Phule G-12, ILC 'O' and L-550 collected from All India Pulses Improvement Project, MPKV, Rahuri.

Table 1: Evaluation of chickpea lines against *F. oxysporum* f. sp. *ciceri* under wilt sick soil.

Name of genotypes	Wilting %	Reaction
WR 315	0	Immune
Digvijay	2.71	Resistant
Vishal	3.84	Resistant
Virat	5.28	Resistant
Vihar	8.72	Resistant
Vijay	6.18	Resistant
Vikas	30	Susceptible
Vishwas	42.6	Highly Susceptible
Phule G-12	54.22	Highly Susceptible
ILC 'O'	81.75	Highly Susceptible
L550	83.30	Highly Susceptible
JG 62	100	Highly susceptible

Raising of seedlings for experiments

Seeds of Six resistant and six susceptible lines were soaked overnight in sterilized water. Soaked seeds were sown separately in plastic pots containing sterilized sand soil mixture. Seedlings were raised up to 15 days while watering with sterilized water.

Observations for wilt incidence

For this experiment, six resistant and six susceptible lines were raised and inoculated in plastic pots. Number of plants showing yellowing and drooping symptoms were recorded from 15 days after inoculation (DAI) and observations were taken frequently up to 45 DAI. The percentage of wilt incidence was calculated and the lines under testing were categorized as the score of 0 for no symptoms, 1 for <1% mortality of plants, 3 for >1 to 10% mortality of plant and 5 for 11 to 20% mortality, 7 disease score for 21 to 50% mortality and 9 score for >50% plant mortality. Lower disease incidence with 3 disease score can be taken as resistance source (Reddy et al., 2009).

Root anatomical study

Fifteen days old seedlings were uprooted and washed properly. Transverse sections were taken from roots of healthy seedlings. For each line, tap root of five uniform seedlings were taken for making sections and at least ten cross sections were observed under light microscope. Observations were taken on diameter of vascular bundle, protoxylem and metaxylem number, protoxylem and metaxylem diameter using ocular micrometer under light microscope (BX-50, Olympus stereo-binocular microscope with photo-graphic attachment at magnification 10X of objective lens. (Pandey et al., 1997)

Counting of secondary lateral roots

In a separate experiment, seed germination was carried out on Petri plates using soaked blotting paper. After 7 days, morphological differences especially with respect to number of lateral roots and length of tap root (cm) were recorded to compare the Fusarium wilt resistant and susceptible lines of chickpea.

Data analysis

Based on the number and diameter (μm) of protoxylem and metaxylem, total xylem area were calculated for comparison of resistant and susceptible lines. These parameters were analyzed using scattered plot for categorizing the resistant and susceptible groups of chickpea genotypes.

RESULTS

Six resistant and six susceptible lines have been tested for wilt in wilt sick soil in pot culture and wilt incidence was recorded. The chickpea genotype WR 315 shown immune reaction and Digvijay and Vishal have shown minimum wilt incidence of 2.71 % and 3.84 %. Wilt incidence in Virat, Vihar and Vijay was comparatively higher 5.28 % to 8.72 % while susceptible lines have shown above 30 % wilt incidence. Therefore, wilt incidence below 10 was categorized as resistant and that above 10 as susceptible.

Table 2: Root morphological features in wilt resistant and susceptible lines of chickpea.

Name of genotypes	Average No. of lateral roots	Average length of tap root (cm)
WR315	10.6	9.2
Digvijay	15.8	9.3
Vishal	12.6	9.6
Virat	14.8	6.4
Vihar	12.0	7.8
Vijay	12.6	6.5
S.E $\pm$	1.98	0.66

C.D. at 5%	5.82	1.93
JG62	24.0	4.7
Vikas	23.5	7.4
Vishwas	17.8	8.2
Phule G 12	23.8	6.5
ILC 'O'	20.5	5.8
L 550	21.2	6.8
S.E.	3.38	1.20
C.D. at 5%	10.18	3.84

Root morphological features

The 15 days old seedlings of chickpea genotypes were used for root morphological studies. In majority of the susceptible lines, observed number of lateral roots to be higher as compared to resistant lines (Table 2). In the susceptible group lateral root number varied from 17.8 to 24.0 and the highest number was observed in JG 62. In resistant group, number varied from 10.6 to 15.8 with the minimum being in WR 315 and the maximum in the line Digvijay having the lateral root number 15.8. The length of tap roots found to be higher in wilt resistant genotypes as compared to susceptible genotypes. In the resistant group average length of tap roots ranged from 6.4 to 9.6 cm and the highest length was observed in Vishal and minimum in Virat. In susceptible group, number varied from 4.7 to 8.2 cm with the minimum being in JG 62 and the maximum in the line Vishwas.

Root anatomical features

Transverse section (TS) of healthy tap roots of resistant and susceptible lines were observed microscopically for counting vascular elements (Table 3). Additionally, protoxylem, metaxylem and total vascular diameter were measured. Marked difference between resistant and susceptible lines was observed with reference to protoxylem and metaxylem number and metaxylem diameter. Resistant lines have a common feature of larger number of protoxylem and metaxylem as compared to susceptible lines. Protoxylem number in resistant lines has been in the range of 70 to 104 with WR 315 recording the highest number 104 as compared to the range of 60 to 95 in susceptible lines. Metaxylem number in resistant lines varied between 41 to 66 and highest being in WR 315 (66) as compared to 35 to 58 in susceptible lines.

Table 3: Vascular elements of wilt resistant and susceptible lines of chickpea.

Sr.No	Name of the lines	Average Vascular Bundle Diameter (µm)	Average number Proto xylem	Average number Meta Xylem	Average diameter Meta xylem (µm)	Average diameter Proto xylem (µm)	Total cross section area (µm ²)
1.	WR315	670	104	66	67.20	23.00	21085.72
2.	Digvijay	460	102	50	60.00	28.00	20776.75
3.	Vishal	560	90	41	51.20	24.60	17912.56
4.	Virat	532	95	65	58.00	24.80	18751.47
5.	Vihar	630	70	46	66.00	28.00	19037.62
6.	Vijay	570	100	45	54.00	30.00	20648.37
	SE±	6.84	4.46	3.71	3.39	2.66	
	CD at 5%	19.98	13.02	10.83	9.90	7.98	
7.	JG62	190	60	35	26.40	13.20	10735.57
8.	Vikas	207	75	51	34.80	21.60	12595.27
9.	Vishwas	330	95	58	32.20	16.40	14726.5
10.	Phule G 12	250	70	46	30.00	15.00	11486.27
11.	ILC 'O'	370	74	45	55.4	21.80	13602.48
12.	L 550	220	69	35	26.40	15.00	12709.71
	SE±	6.50	3.89	3.63	2.50	1.88	
	CD at 5%	18.98	11.35	10.60	7.32	5.50	



Fig 1: Root morphological (Length of tap root and No.of lateral roots) study of chickpea genotypes. A) Fusarium wilt Susceptible genotypes JG 62, B) Fusarium wilt Resistant genotype WR 315, C) Cultivation of chickpea on raised bed for root anatomical studies.

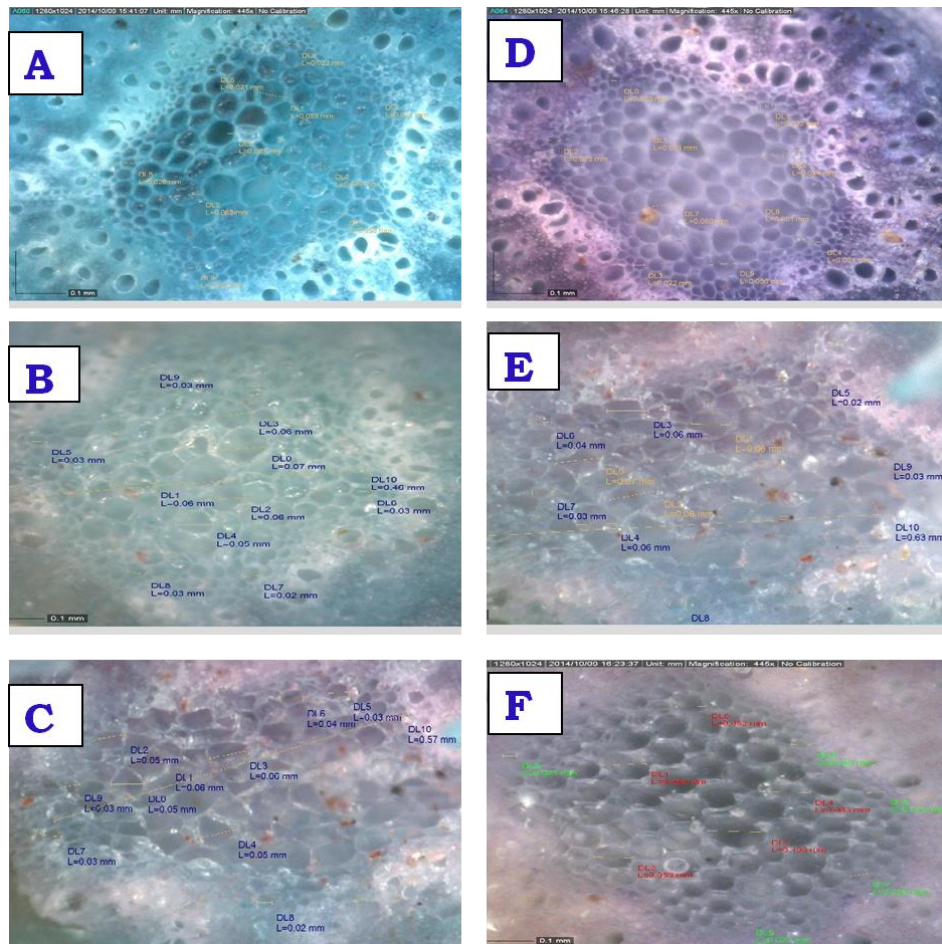


Fig: 2 Root anatomical view of wilt resistant lines of chickpea (A) WR 315, (B) Digvijay, (C) Vijay, (D) Vishal, (E) Vihar, (F) Virat

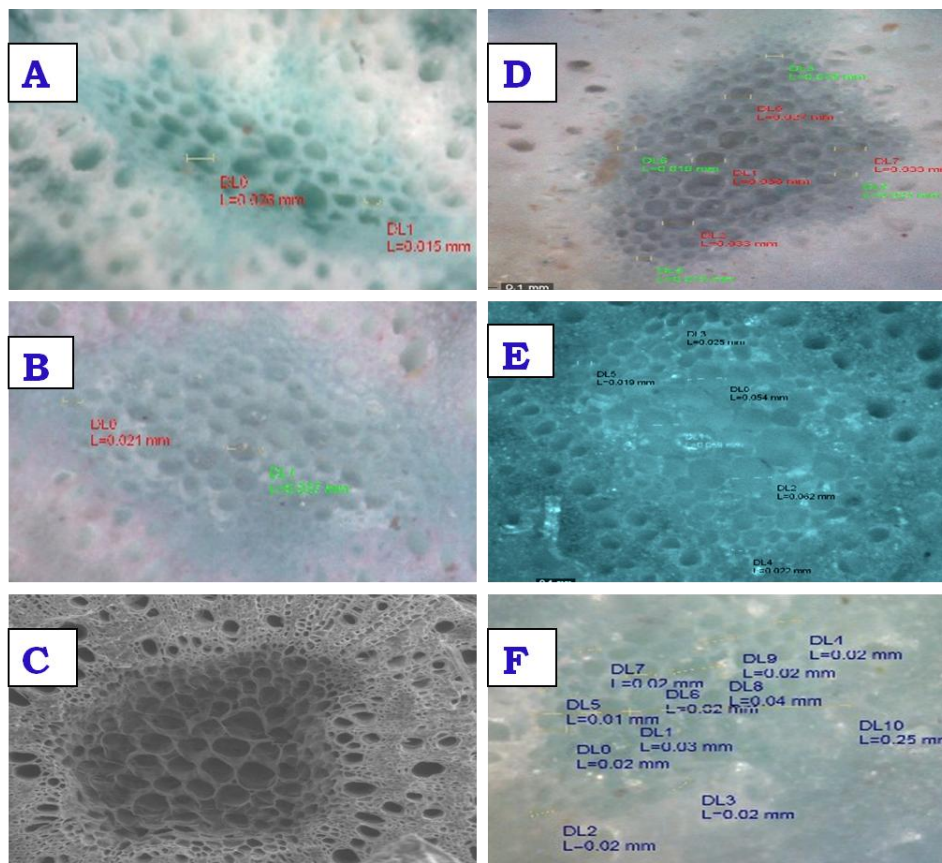


Fig 3. Root anatomical study of wilt susceptible lines of chickpea: A) JG 62, B) Vikas, C) Vishwas, A) Phule G-12, B) ILC 'O', C) L550

The protoxylem diameter has shown remarkable variation than the metaxylem diameter in resistant and susceptible lines of chickpea. The metaxylem diameter in resistant genotypes varies from 51.20 to 67.20 μm and it is highest in WR 315 (67.20) however in susceptible lines metaxylem diameter varies from 26.40 to 55.40 μm . The least metaxylem diameter was observed in JG 62 and L 550 (26.40 μm) comparatively higher in ILC 'O' genotype of susceptible group. (55.40 μm). The protoxylem diameter in resistant genotypes in the range of 23.00 to 30.00 μm and it was highest in genotype, Vijay (30.00 μm) where as in susceptible lines diameter of protoxylem varies from 13.20 to 21.80 μm . The least protoxylem diameter was observed in JG 62 (13.20 μm) and higher in ILC 'O' genotype of susceptible group. (21.80 μm). In the resistant group of genotypes, number as well as diameter of protoxylem and metaxylem was higher than the susceptible genotypes. Vascular bundle diameter has been found to vary across the length of tap-root. However, number and diameter of the protoxylem and metaxylem has been seen to be a consistent character within 2 cm below the collar region of seedlings. Therefore, only protoxylem and metaxylem number and diameter has been taken into consideration for the analysis disregarding the vascular bundle diameter. The present finding is supported by earlier observations.

Saxena and Khare (1988) reported that lentil genotype with lesser wilt had significantly shorter or less number of secondary lateral roots. Lateral root number was recorded for resistant and susceptible lines of pigeonpea but tissue characteristics were not consistent with resistant and susceptible plant. It is possible that chances of pathogen entry increases with the increase in the number of lateral roots. Cross section has also been found to be important distinguishing criteria between resistant and susceptible lines. Total xylem bundle area (protoxylem + metaxylem area) have been found to be consistently larger in resistant than in susceptible lines. (Table-3). Average vascular bundle diameter (μm) was found to be higher in resistant genotypes (460 to 670 μm) than the susceptible genotypes (190 to 370 μm). However, categorization or grouping of resistant and susceptible lines of chickpea on the basis of vascular elements. Based on the data on vascular elements, there seems to have a correlation between higher protoxylem number and total xylem area and these two variables could differentiate between the resistant and susceptible lines of chickpea. When protoxylem number and total xylem area have been plotted, it has been found that resistant group tends to have higher number of protoxylem and larger total xylem area seems to be the better discriminator than other vascular features (Fig.-1). Pandey et al. (1997) have shown xylem vessels in root and stem are clogged and browning occurs in susceptible types as compared to little or no browning in resistant lines. In both susceptible and resistant type initial infection is similar; however, wilt symptom express in susceptible type only. It was suggested that localization of the pathogen occurs in resistant type (Beckman, 1987). It can be considered as a good method of classification between resistant and susceptible genotypes of chickpea.

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