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EVALUATION OF CHICKPEA GERMPLASM FOR RELATIVE RESISTANCE OR SUSCEPTIBILITY AGAINST FUSARIUM WILT AND ASCOCHYTA BLIGHT UNDER FIELD CONDITIONS

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ABSTRACT

Recognition and use of resistant sources against pests and diseases are an integral element of a genetic improvement program. For this purpose, an evaluation of chickpea cultivars (24 advanced lines and 6 commercial varieties) was undertaken under field conditions. Three types of disease responses based on a disease rating scale of 1-9 were observed i.e. resistant, moderately resistant, and susceptible. It was noticed that among 30 cultivars, none was highly resistant and asymptomatic or highly susceptible to both diseases. It was remarkably noticed that in the case of *Fusarium* wilt, all of the chickpea genotypes except one which performed better was categorized as resistant. Contrarily, L124 was scored as moderately susceptible. All the commercial varieties NUT (2018-19), DG-92, Rabat, Black gram, Benezir, and Synyasi were susceptible to *Fusarium* wilt. In the case of *Ascochyta* blight, all the germplasm exhibited resistant reactions except one (L124). Out of the six commercial varieties, Black gram and Benezir exhibited resistant reactions. *Fusarium* wilt and *Ascochyta* blight gradually increased with time after each observation. *Fusarium* wilt disease index in the month of March, was significantly higher on commercial varieties, including NUT (2018-19), DG-92, Rabat, Benezir, and Synyasi, ranging between 57.4-61.7% followed by cv. Black gram with a disease index of 53.7%. All the advanced lines had a low disease index as compared to commercial varieties. Similarly, in the month of March, the disease index of *Ascochyta* blight was lowest on L102 (38.9%) and the highest on Nut-2018-19 (59.3%) followed by Rabat and Synyasi. Significantly maximum 1000 grain weight was recorded in DG-92, L10, and L119, ranging from 304.3-305.3 g. In terms of grain yield/hectare, L117, L124, NUT (2018-19) and Black gram produced a significantly maximum yield (2916.7-2868.1 kg/ha) followed by Rabat (2638.9 kg/ha) and Synyasi (2520.8 kg/ha) whereas, the lowest yield was recorded in L121. The disease severity of both diseases was positively correlated with 100-grain weight as well as with grain yield. The study revealed the availability of resistant germplasm against two important diseases (*Fusarium* wilt and *Ascochyta* blight) which may be exploited in the breeding program for the development of disease-resistant cultivars and may be incorporated with high-yielding cultivars which are clearly evident in the present study.

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INTRODUCTION

Pulses are important leguminous food crops and vital source of food (Malik et al., 2011). The chickpea is cultivated all around the world and plays a magnificent role in Pakistan agriculture. It is cultivated over fifty countries worldwide and rank 3rd in leguminous production (FAOSTAT, 2014). It is an inexpensive, good and essential source of protein (Rasool et al., 2015). It also contains fat, fiber, and other carbohydrates (Grasso et al., 2021). Due to its medicinal properties, the chickpea is useful in many diseases. The annual world production of chickpea is 11.62 million tons and average yield production is 1.4 tons/ha globally (FAOSTAT, 2012; ICRISAT, 2014). The chickpea crop accounts for 15 percent in entire world pulses production (Shakoor et al., 2015). The yield in Pakistan is very poor (7573.19 Hg/Ha) as compared to the high yielding country like Israel (61197.7 Hg/Hg) (Shahzaman et al., 2016). Among biotic stresses, blight and wilt of chickpea are the most serious and are non-linear in comparison.

Fusarium wilt outbreak can be destructive to crops and cause up to 100% loss under helpful environment (Jendoubi et al., 2017) while 10-15% yield losses are regular features by *Fusarium* wilt (Campbell and Madden, 1990). In Pakistan, this disease caused 10-50% losses each year (Nazir et al., 2012). Disease symptoms can appear at any point of plant growth; in susceptible germplasm symptoms appeared within 25 days of sowing as early wilt (Al-Taae et al., 2013; Jiménez-Díaz et al., 2015). However, symptoms of wilt mostly detectable in the initial stage of flowering and can be visible up to podding stage that is classified as late wilt.

Ascochyta rabiei is the causal organism of *Ascochyta* blight, a highly destructive disease, which reduces yield as well as quality of crop (Sarwar et al., 2012; Sharma and Ghosh, 2016). Significant yield losses have been reported in India, Pakistan, Australia, Morocco, Spain, Syria, USA, Iran and Canada (Gayacharan et al., 2020). In Pakistan, epidemics of *Ascochyta* blight are responsible for huge losses, and in normal conditions cause 20-25% losses in yield each year by this disease (Jamil et al., 2010). Symptoms of *Ascochyta* blight appear on all aerial parts of the plants. Lesion first appear as gray on the leaves, stems or pods and turn into brown lesions with borders become dark. Disease develops under favorable conditions and then these spots become larger swiftly and coalesce, the leaves and buds become blight (Nene et al., 2012).

There are many different methods to control diseases viz. use of resistant varieties, cultural practices, pesticides and biological control agents. While each one of these strategies has their own importance, when applied alone for control of disease, none of them is completely successful yet (Chandel and Deepika, 2010). Hence, the low-priced and most efficient management strategy against chickpea diseases is the use of tolerant varieties under such conditions. Although, efforts have been made to developed resistant and high yielding varieties (Ali et al., 2002.; Ghazanfar et al., 2010). Current investigation was carried out to screen the chickpea germplasm for identifying resistance sources against Fusarium wilt and Ascochyta blight under field conditions.

MATERIAL AND METHODS

A field experiment was conducted at Pulses Research Sub-Station, Agriculture Research Institute, Tandojam to screen the chickpea germplasm, including commercial varieties and advanced lines against Fusarium wilt and Ascochyta blight as well as to determine their effects on yield.

Experimental details

After land preparation, the seeds of twenty four chickpea advanced lines viz. L101, L102, L103, L104, L103, L105, L106, L107, L108, L109, L110, L111, L112, L113, L114, L115, L116, L117, L118, L119, L120, L121.L122, L123, L124 and six commercial varieties such as NUT(2018-19), DG-92, Rabat, Black gram, Benezir and Synyasi were planted in lines by single counter hand drill method in an experimental plot arranged as Randomized Complete Block Design and three replication. The row to row distance was kept at 30 cm, plant to plant distance was 10 cm, while the total plot size was 4.8 m^2. All the phosphorus, potassium and 1/3 nitrogen fertilizers were applied at the time of sowing. But, no plant protection measure was applied, as screening of chickpea genotypes for the above mention diseases were planned under natural disease pressure.

Cultivars response to Fusarium wilt and Ascochyta blight

Six plants of all the cultivars were selected randomly from each replication and tagged. The host response to targeted diseases was observed after 30 days of plant emergence in the month of January. Consequently, 2nd and 3rd disease scoring was recorded after 60 and 90 days of emergence in the month of February and March, respectively. Disease scoring was recorded using a 1–9 rating scale similar to those utilized by Jan and Wiese (1991), Chen and Muehlbauer (2003), Sharma et al. (2005) and Pande et al. (2011) (Table 1). Data on plant disease ratings for each replication were averaged. Average disease ratings of three replications were statistically analyzed to generate a mean disease rating for each genotype and to formulate statistical homogenous grouping (LSD at alpha 0.05) using computer software 'Statistix version 8.1'. On the basis of average disease score, the varieties were categorized into reaction type by using the 1-9 rating scale as described by Reddy and Nene (1979). The genotypes rated 1-3 were resistant, 4 moderately resistant, 5-6 susceptible and 7-9 were observed highly susceptible. The observed disease rating for the month of March used to calculate the disease index using the following formula:

Disease Index (%) = [(Sum of numerical rating / No. of observations) / Max. rating] X 100

To determine the effects of these diseases on the grain yield, thousand seed weight (g) was obtained by weighing 1000 seeds using an electronic weighing scale for each genotype. The total yield (Kg/ha) was also calculated by weighing dry grains at 12% moisture from each genotype. Thousand seed weight (g) and total yield (Kg/ha) of three replications from each genotype was statistically analyzed to generate means for all cultivars and to formulate statistical homogenous grouping (LSD at alpha 0.05) using computer software 'Statistix version 8.1'.

RESULTS

Fusarium wilt and Ascochyta blight were identified on the basis of symptoms. The symptoms of Fusarium wilt included dropping of petioles, rachis and leaflets accompanied with yellowing and necrosis of leaves (Figure 1). The symptoms of Ascochyta blight appeared on all aerial parts of the plants as lesions first appear as gray on the leaves, stems or pods and turn into brown lesions with dark borders. Later, in the center of the lesions, the small, round/ disc shape, brown-black dots grow and often organized in homocentric circles and look like a bull's-eye (Figure 2).

Figure 1: *Ascochyta* blight symptoms on chickpea plant.

Figure 2: *Ascochyta* blight symptoms on chickpea pods.

Chickpea cultivars response to Fusarium wilt and Ascochyta blight

The results from the analysis of variance revealed considerable variation towards disease response among chickpea genotypes. Three types of disease responses based on disease rating scale of 1-9 were observed, i.e. resistant, moderately resistant and susceptible. It was noticed that among 30 cultivars, none was highly resistant or asymptomatic or highly susceptible against both the diseases. It is remarkably noticed that in case of Fusarium wilt, all of the chickpea genotypes except one performed better and categorized as resistant with the average disease rating range between 2.7 and 3.4 whereas, L124 was scored as moderately susceptible with the disease rating of 3.9. All the commercial varieties NUT (2018-19), DG-92, Rabat, Black gram, Benezir and Synyasi were susceptible with disease rating ranging between 4.8 and 5.6. As mentioned earlier that same plants were also assessed for Ascochyta blight. All the germplasm exhibited resistant reaction except one (L124). Same cultivar was found as moderately susceptible for Fusarium wilt; whereas, in case of Ascochyta blight it became susceptible with an average disease rating of 4.7. Out of the six commercial varieties, NUT (2018-19), DG-92, Rabat, Black gram, Benezir and Synyasi (two of the varieties i.e., Black gram and Benezir) exhibited resistant reactions with an average disease rating of 4.4 and 3.8 respectively. On the other hand, the remaining four varieties were found susceptible having 4.9-5.2 disease rating (Table 1).

Table 1: Disease rating scale (1–9) used for the assessment of *Fusarium* wilt and *Ascochta* blight.

Disease Rating	Disease Symptoms	Host Response
1	No visible symptoms	Resistant
2	Minute lesions prominent on the apical stem	Resistant
3	Lesions up to 5-10 mm in size and slight drooping of apical stems	Resistant
4	Lesions obvious on all plant parts and clear drooping of apical stem	Moderately Resistant
5	Lesions on all plant parts, defoliation initiated, drying and breaking of branches slight to moderate	Susceptible
6	Lesions as in 5, defoliation, dry, broken branches common, some plants killed	Susceptible
7	Lesions as in 5, defoliation, dry, broken branches very common, up to 25 % of plants killed	Highly Susceptible
8	Symptoms as in 7 but up to 50 % of the plants killed	Highly Susceptible
9	Symptoms as in 7 but up to 100 % of the plants killed	Highly Susceptible

Disease index of *Fusarium* wilt and *Ascochyta* blight

In the present study, *Fusarium* wilt and *Ascochyta* blight gradually increased with time after each observation. The disease index of *Fusarium* wilt at 1st observation ranged from 19.8-32.1%. It increased in the 2nd observation to 22.8-56.8%, which gradually increased and ranged 30.3-61.7% at the time of third observation. Similarly, the disease index of *Ascochyta* blight at the time of 1st observation ranged from 19.8-38.3%, which increased at 2nd observation to 23.5-54.3%. The highest disease index of *Ascochyta* blight observed at the time of third observation was 38.9-59.3% (Figure 3 and 4).

Disease index recorded in the month of March (after three months of emergence) statistically analyzed (LSD at alpha 0.05) for homogenous grouping. Fusarium wilt disease index was significantly. Commercial varieties, including NUT (2018-19), DG-92, Rabat, Benezir and Synyasi was grouped as a with highest disease index, ranging between 57.4-61.7% followed by. cv. Black gram with disease index of 53.7%. All the advanced lines had low disease index as compared to commercial varieties. Significantly, lowest disease index was recorded in L101, L107, L108, L111 and L116, ranges between 32.1-31.5% (Figure 5).

Disease index ratings of *Ascochyta* blight revealed that all the genotypes statistically differed in terms of *Ascochyta* blight. The lowest disease index of 38.9% was recorded in L102 and the highest 59.3% was recorded in Nut-2018-19, followed by Rabat and Synyasi 57.4 and 56.2, respectively. Among the commercial varieties, lowest disease index of 42.0% for *Ascochyta* blight was recorded in Benezir (Figure 6).

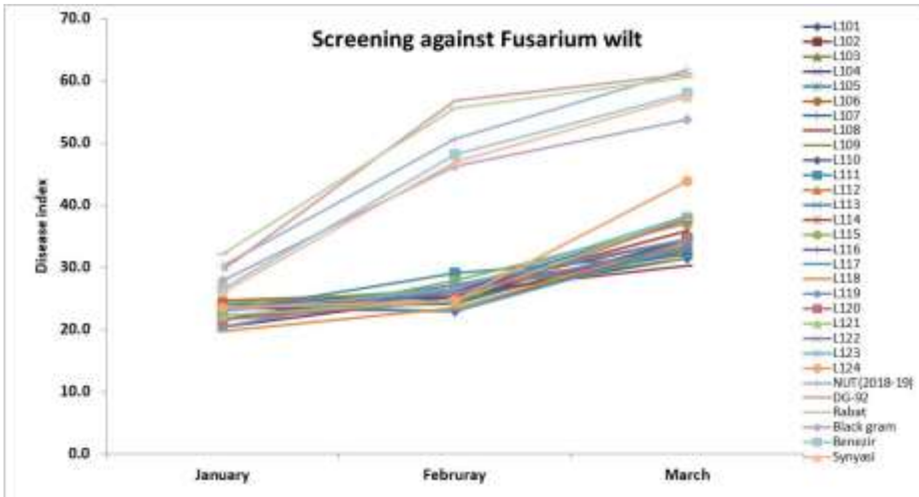

Figure 3: Disease index of thirty different germplasm/varieties of chickpea against *Fusarium* wilt at three different time point.

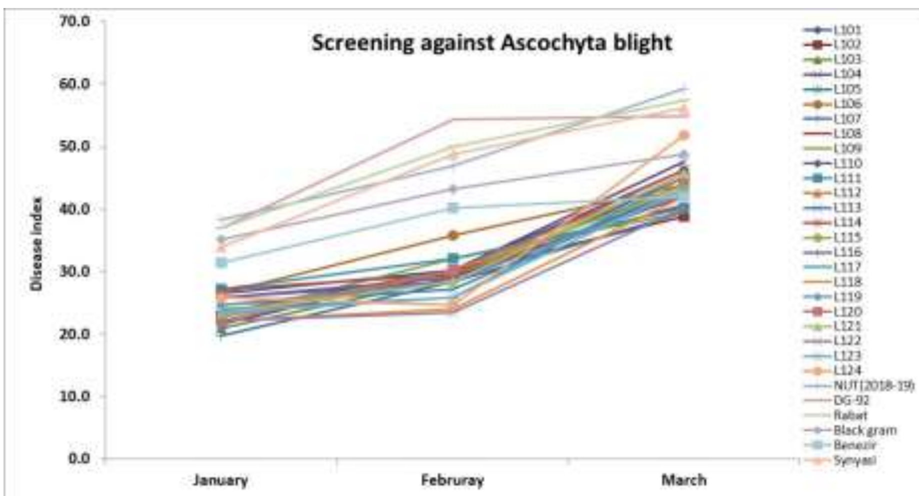

Figure 4: Disease index of thirty different cultivars of chickpea against *Ascochyta* blight at three different time point.

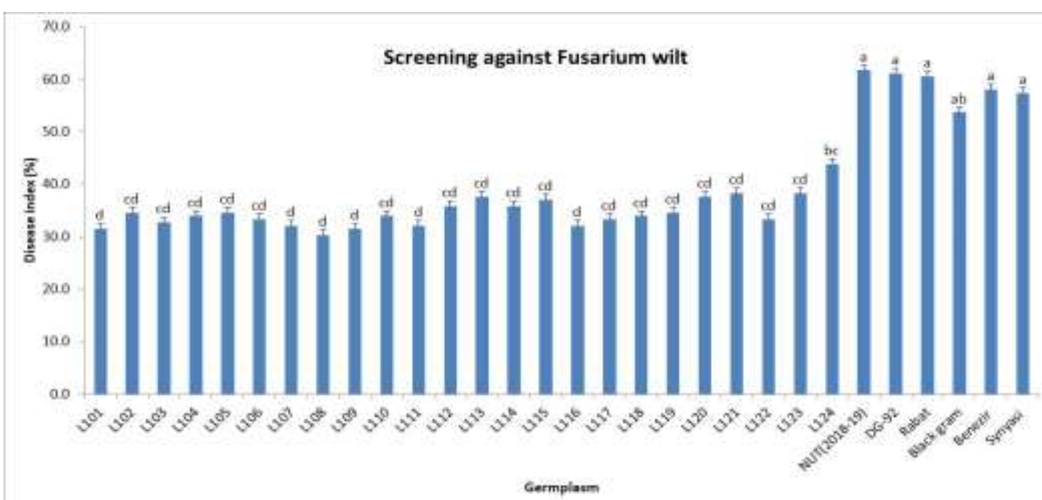

Figure 5: Disease index of thirty different cultivars of chickpea against *Fusarium* wilt in the month of March.

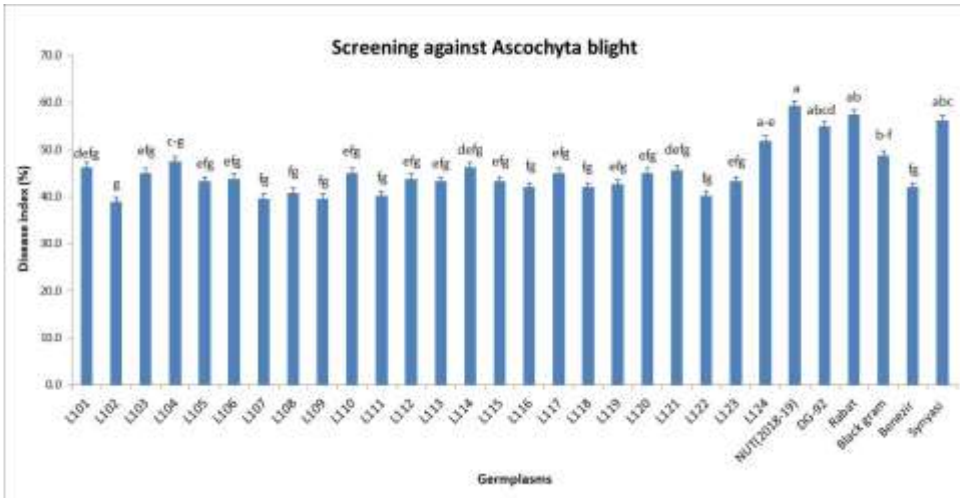

Figure 6: Disease index of thirty different cultivars of chickpea against *Ascochyta* blight in the month of March.

Grain yield

At harvesting, yield of each genotype was calculated to evaluate their performance. Two advanced lines (L117 and L124) and two commercial varieties NUT (2018-19) and Black gram produced a significantly maximum yield (2916.7-2868.1 Kg/ha) followed by Rabat (2638.9 kg/ha) and Synyasi (2520.8 kg/ha). On the other hand, lowest yield was recorded in L121 (1041.7 Kg/ha) (Figure 7).

To consider the quality of grains produced 1000 grain weight (g) was also assessed. One commercial variety (DG-92 and two advanced lines (L10 and L119) produced significantly maximum 1000-grain weight ranging from 304.3-305.3 g. The 2nd lowest 1000-grain weight was recorded in remaining five commercial varieties NUT (2018-19), Rabat, Black gram, Benezir and Synyasi and four advanced lines (L105, L113, L118 and L124) ranging from 292.3-292.7 g. On the other hand, significantly minimum 1000-grain weight was recorded in L103 (Figure 8).

Correlation between yield parameters and disease severity

To find out to what extent variation in disease severity affected the yield, R-squared values were calculated using the computer software "Excell'. R-squared between disease severity of Fusarium wilt and yield found positively correlated (R^2 = 0.36) only 36% variation in yield may be explained by disease severity (Figure 9). Similarly, correlation between 1000 grain weight was calculated which turned out positive, but weakly correlated (R^2 = 0.29) (Figure 10).

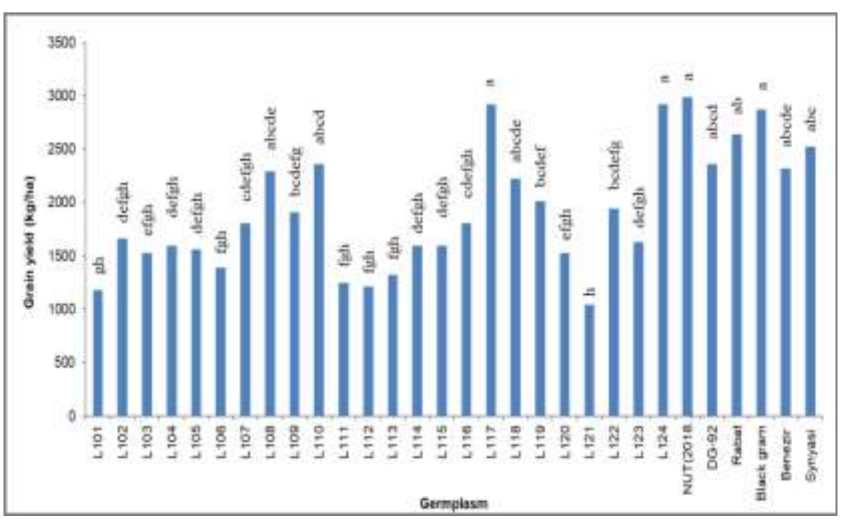

Figure 7: Grain yield (kg/ha) of thirty different cultivars of chickpea.

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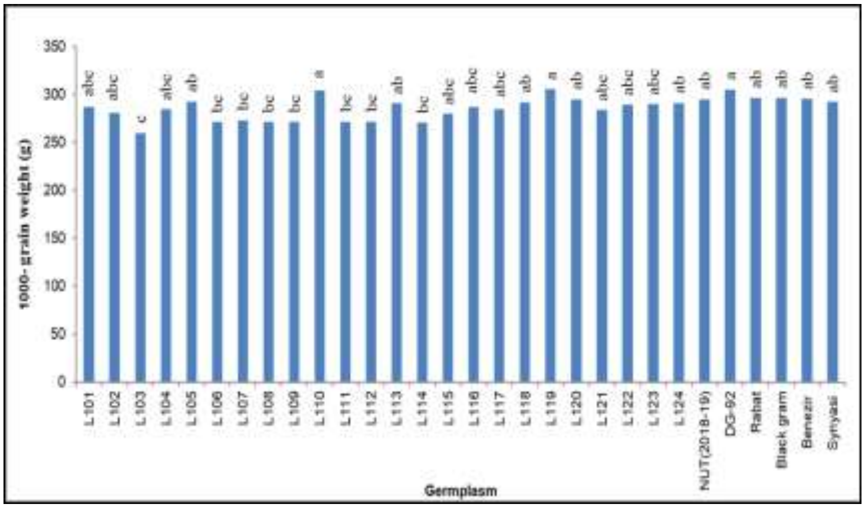

Figure 8: Thousand grain weight (g) of thirty different cultivars of chickpea.

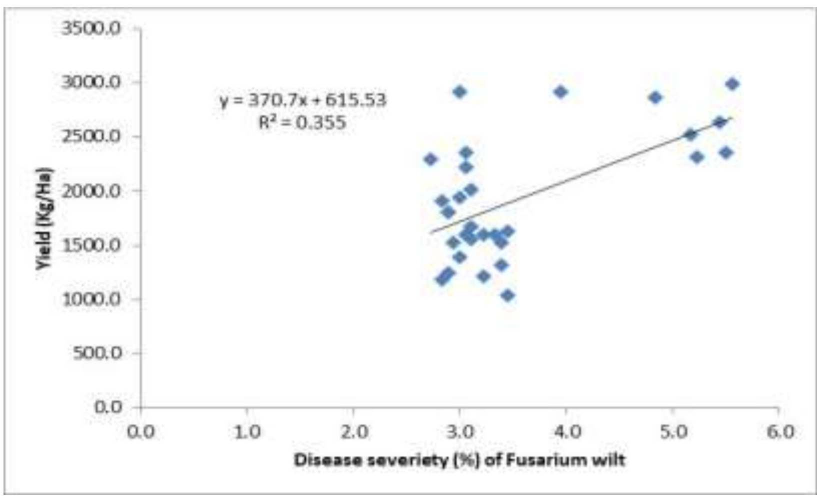

Figure 9: Correlation analysis between yield (Kg/ha) and disease severity of *Fusarium* wilt at thirty different chickpea cultivars.

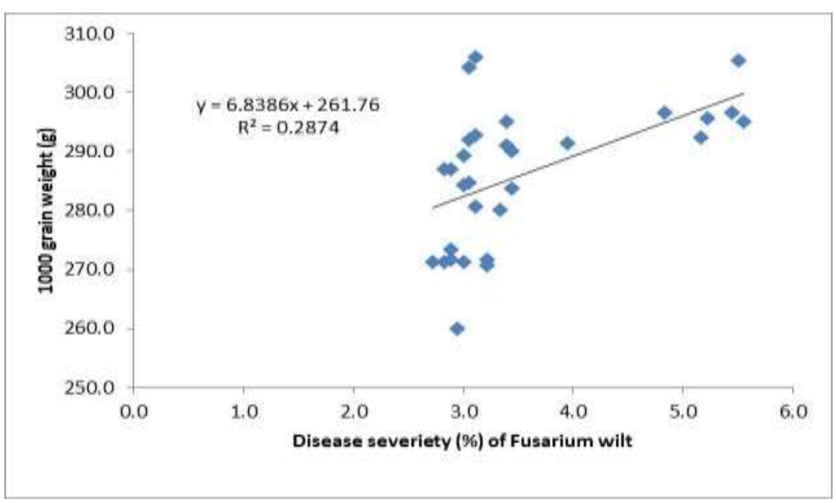

Figure 10: Correlation analysis between 1000 grain weight and disease severity of *Fusarium* wilt at thirty different chickpea cultivars.

In case of Ascochyta blight, R-squared between disease severity and yield as well as between disease severity and 1000 grain weight found positively correlated and recorded as 0.29 and 0.18, respectively (Figure 11 and 12).

DISCUSSION

The results from the analysis of variance revealed considerable variations towards disease response among chickpea genotypes. Three types of disease responses based on disease rating scale of 1-9 were observed i.e. resistant, moderately resistant and susceptible. It was noticed that among 30 cultivars, none was highly resistant and asymptomatic or highly susceptible against both the diseases.

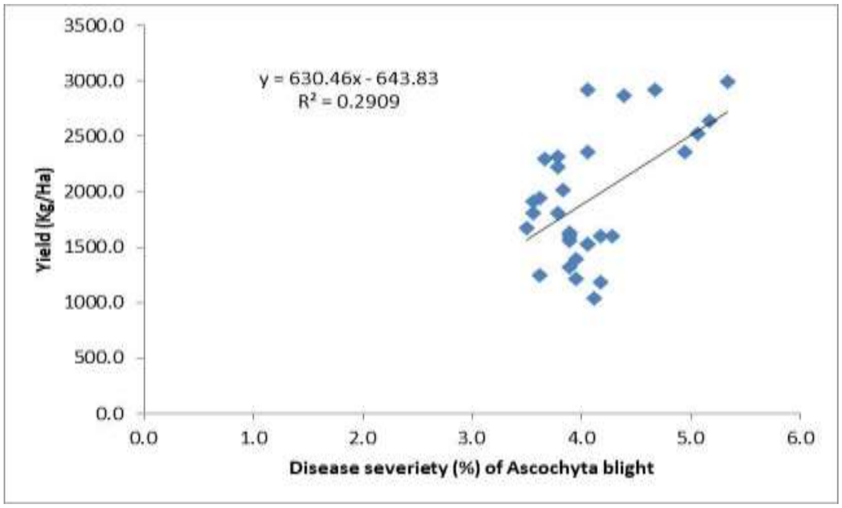

Figure 11: Correlation analysis between yield (Kg/ha) and disease severity of *Ascochyta* blight at thirty different chickpea cultivars.

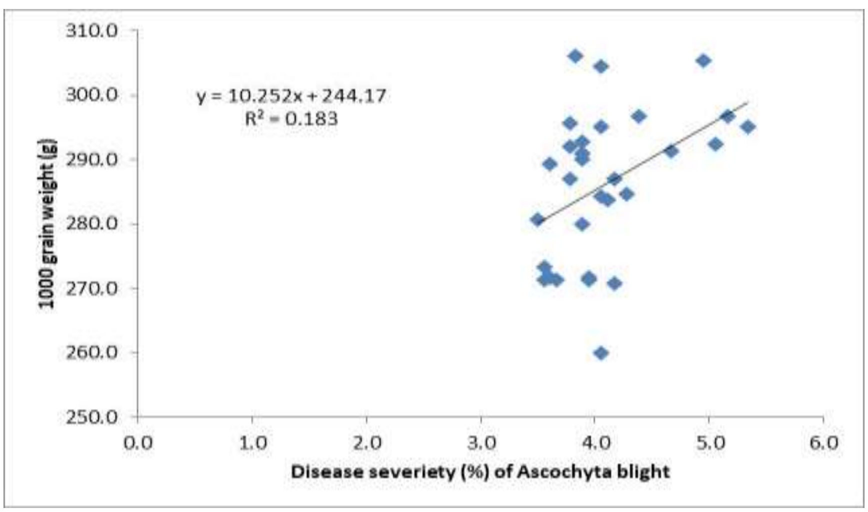

Figure 12: Correlation analysis between 1000 grain weight and disease severity of *Ascochyta* blight at thirty different chickpea cultivars.

It is remarkably noticed that in case of *Fusarium* wilt, all of chickpea genotypes except one performed better and categorized as resistant with the average disease rating range between 2.7-3.4 whereas, L124 was scored as moderately susceptible with the disease rating of 3.9. All the commercial varieties NUT (2018-19), DG-92, Rabat, Black gram, Benezir and Synyasi were found susceptible with disease rating ranging between 4.8 and 5.6. In the present study, *Fusarium* wilt development gradually increased with time after each observation. The disease index of *Fusarium* wilt at 1st observation ranged from 19.8-32.1%. It increased in the 2nd observation to 22.8-

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56.8%, which gradually increases and ranged 30.3-61.7% at the time of third observation. Disease index recorded in the month of March (after three months of emergence) statistically analyzed (LSD at alpha 0.05) for homogenous grouping. *Fusarium* wilt disease index was significantly. Commercial varieties, including NUT (2018-19), DG-92, Rabat, Benezir and Synyasi were grouped as a with highest disease index, ranging between 57.4-61.7% followed by. cv. Black Gram with disease index of 53.7%. All the advanced lines have low disease index as compared to commercial varieties. Significantly, lowest disease index was recorded in L101, L107, L108, L111 and L116, ranges between 32.1-31.5%. Many studies conducted in different parts of the world revealed the same pattern of cultivars response against *Fusarium* wilt. Intizar-ul-Hassan et al. (2011) evaluated 99 accessions and observed that 9 were resistant, 4 moderately resistant, 26 moderately susceptible and 42 susceptible at reproductive stage. In another study, out of 48 cultivars, 16 were highly susceptible, 8 susceptible, 3 moderately susceptible, 10 resistant response and 11 moderately resistant (Ahmed et al., 2013). Similarly, Benzohra-Belaidi (2016) found three resistant cultivars out of 13 chickpea genotypes accessions to *Fusarium oxysporum*. Hotkar et al. (2018) evaluated 31 lines against *Fusarium* wilt and recorded 10 lines resistant, 18 lines moderately resistant, 18 cultivars susceptible and 10 were highly susceptible. Venkataramanamma et al. (2018) tested 85 chickpea cultivars against *Fusarium* wilt for two growing seasons and found that 7 lines appeared resistant, 6 moderately resistant, 26 moderately susceptible, 33 susceptible, while remaining 13 lines were highly susceptible. Similarly, Mahajan et al. (2019) evaluated 82 chickpea genotypes against *Fusarium* wilt during 2016-17 and 2017-18. They observed 4 and 27 genotypes with resistant and moderately resistant reactions, respectively in both years.

In case of *Ascochyta* blight, all the germplasm exhibited resistant reaction except one (L124). Same cultivar was found as moderately susceptible for *Fusarium* wilt; whereas, in case of *Ascochyta* blight it becomes susceptible with an average disease rating of 4.7. Out of the six commercial varieties NUT (2018-19), DG-92, Rabat, Black gram, Benezir and Synyasi two of the varieties i.e. Black gram and Benezir exhibited resistant reactions with an average disease rating of 4.4 and 3.8, respectively. The disease index of *Ascochyta* blight gradually increased with time after each observation. The disease index of *Ascochyta* blight at the time of 1st observation ranged from 19.8-38.3%, which increased at 2nd observation to 23.5-54.3%. The highest disease index of *Ascochyta* blight observed at the time of third observation i.e. 38.9-59.3%. Disease index ratings of *Ascochyta* blight revealed that all the genotypes statistically differed in terms of *Ascochyta* blight. The lowest disease index of 38.9% was recorded in L102 and the highest 59.3% was recorded in Nut-2018-19, followed by Rabat and Synyasi 57.4 and 56.2, respectively. Among the commercial varieties, the lowest disease index of 42.0% for *Ascochyta* blight was recorded in Benezir.

Kiprop (2016) evaluated 25 chickpea cultivars against *Ascochyta* blight and observed that 8 moderately resistant 12 susceptible, 5 highly susceptible, 5 high yielding and moderately resistant. Shah et al. (2021) evaluated 60 elite chickpea lines under manipulated conditions and observed that none of them was resistant whereas, only 8 chickpea desi lines were observed moderately resistant, 3 lines were tolerant, 17 were moderately susceptible and the remaining 32 lines were found susceptible and highly susceptible. Kimurto et al. (2013) screened 36 chickpea cultivars including commercial varieties as well as advanced lines against *Ascochyta* blight disease and found that commercial varieties were more susceptible than advanced lines.

Khan et al. (2018) screened 85 chickpea Kabuli cultivars against Ascochyta blight and observed 9 genotypes to be resistant, 6 lines moderately resistant, while 2 moderately susceptible, 3 lines susceptible, 65 genotypes were highly susceptible. However, no chickpea genotype showed highly resistant against blight disease of chickpea. Grasso et al. (2021) screened out four commercial varieties and 109 genotypes and reported that all genotypes were affected by this fungus, without occurrence of asymptomatic plants. The genotypes ranged from resistant to highly susceptible. According to disease reaction, the genotypes were classified as: resistant (2.75%), moderately resistant (32.09%), susceptible (60.55%) and highly susceptible (1.83%).

At harvesting, yield of each genotype was calculated to evaluate their performance. Two advanced lines (L117 and L124) and two commercial varieties NUT (2018-19) and Black gram produced a significantly maximum yield (2916.7-2868.1 Kg/ha) followed by Rabat (2638.9

kg/ha) and Synyasi (2520.8 kg/ha). Whereas, lowest yield was recorded in L121 (1041.7 Kg/ha). To consider the quality of grains produced 1000 grain weight (g) was also assessed. One commercial variety (DG-92 and two advanced lines (L10 and L119) produced significantly maximum 1000-grain weight ranging from 304.3-305.3 g. The 2nd lowest 1000-grain weight was recorded in remaining five commercial varieties NUT (2018-19), Rabat, Black gram, Benezir and Synyasi and four advanced lines (L105, L113, L118 and L124) ranging from 292.3-292.7g. On the other hand, significantly minimum 1000-grain weight was recorded in L103. To find out to what extent variation in disease severity affected the yield, R-squared was calculated using the computer software "Excell'. R-squared between disease severity of Fusarium wilt yield found positively correlated (R^2 = 0.36) only 36% variation in yield may be explained by disease severity. Similarly, correlation between 1000 grain weight was calculated which turn out positive, but weakly correlated (R^2 = 0.29). In case of Ascochyta blight, R-squared between disease severity and yield as well as between disease severity and 1000 grain weight found positively correlated and recorded as 0.29 and 0.18, respectively. Similarly, Khan et al. (2011) evaluated 47 lines of chickpea and found significant variation in terms of 100 seed weight, grain yield/plant and biological yield/plant. Likewise, Mallu (2015) conducted a comprehensive screening of 58 Desi and 37 Kabuli genotypes for desirable yield parameters. According to overall means the results showed a wide range of differences among germplasm with respect to seed yield/ ha, ranging from 0.4-1.3 ton/ha.

CONCLUSION

It is concluded from the present studies that disease severity of both the diseases was positively correlated with 100-grain weight as well as with grain yield. The study revealed the availability of resistant germplasm against two important diseases (*Fusarium* wilt and *Ascochyta* blight) which may be exploited in the breeding program for the development of disease-resistant cultivars and may be incorporated with high-yielding cultivars which are clearly evident in the present study.

AUTHORS' CONTRIBUTION

NK, OI, AB, and SZ designed, planned and prepared layout of the study, NK and OI conducted greenhouse experiments and recorded the data, NK, OI and MT compiled and organized the data, NK, OI and AR analyzed the data, AR made the graphs, all the authors helped in manuscript write up and formatting and NK, OI, AB and MT proofread the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

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