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Response of Chickpea (*Cicer arietinum* L.) Genotypes against Collar Rot Disease Caused by *Sclerotium rolfsii* Sacc.

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Authors' contributions

This work was carried out in collaboration between both authors. All authors' contributed to the study concept and design. All preparations for conducting experiment, monitoring and data collection, analysis was done by author VDB under the guidance of author KN. The draft of manuscript written by author VDB and review, proof reading performed by author KN. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: Collar rot is a fast spreading and destructive disease and is becoming more serious at seedling stage causing rot at collar region especially in area where paddy based cropping system is followed. Lack of sources of resistant in present cultivar against *Sclerotium rolfsii* cause serious threat to chickpea production. Therefore, the present study was carried out to evaluate the chickpea genotypes against *S. rolfsii* to the identification of resistant sources for further breeding program.
Study Design: Randomized Block Design (RBD) with two replications were used.
Place and Duration of Study: Regional Rice Research Station, Navsari Agricultural University, Vyara, between October 2019 to April 2020.
Methodology: Pathogen was isolated from infected collar region of chickpea plant by directly transfer of sclerotia and infected bits on potato dextrose agar (PDA) medium. After purification,

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pathogen was identified by observed the colony character and sclerotia formation. The morphological characters *viz.*, mycelial growth and mycelial characteristic were studied under high power magnification (40X) and sclerotia formation, shape and colour were studied under low power magnification (10X) from 10 days old culture. 39 chickpea genotypes were used for the studies. A field experiment was conducted during *Rabi* -2019-20 season. The inoculum was thoroughly broadcasted in soil @ 10g/ row. Germination per cent and disease incidence were observed.

Results: Chickpea (*Cicer arietinum* L.) collar rot disease caused by *Sclerotium rolfsii* Sacc. was exhibited initial white fluffy mycelium appearance on potato dextrose agar medium. Microscopic view of mycelium was hyaline, branching, compact with septate and had a clamp connection. White sclerotia were formed after 4th days of incubation and later within 10 days after incubation it became mature and colour changed from brown to dark brown. Sclerotia appeared shiny due to presence of gummy material. Out of 39 chickpea genotypes only five *viz.*, GJG-1713, GG-6, GJG-1509, Phule Vikram and JGK-1 were identified as moderate resistant against collar rot disease caused by *S. rolfsii* under inoculums inoculated rice fellow cropping system.

Conclusion: Chickpea genotypes *viz.*, GJG-1713, GG-6, GJG-1509, Phule Vikram and JGK-1 were showed moderate resistant reaction against collar rot disease caused by *S. rolfsii.*

Keywords: Chick pea; collar rot; genotypes; mortality; resistant; Sclerotium rolfsii.

1. INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the world's third most important food legume crop after dry bean and dry pea. Chickpea is also known as Gram, Garbanzobean, Spanish pea, Bengal gram and Chana. Firstly, it was cultivated in South-Eastern areas of the world but now it is also cultivated in semi-arid regions [1]. Chickpea is an important source of protein enriched human food and animal feed, particularly for the low-income population of South-East Asia [2]. In India, chickpea is generally grown as a rainfed crop in the *Rabi* season. Sometimes, it is also grown as a regularly or partially irrigated crop. In India, it occupies an area of 10.56 million hectares and its production is 11.37 million tones with an average productivity of 1078 kg/ha [3]. Whereas, in Gujarat area under chickpea is 2.93 lakh ha with 3.76 lakh tons production with an average productivity of 1285 kg/ha [3].

Despite the high total production, a yield of chickpea is low due to many biotic and abiotic constraints. Among the biotic constraints more than 172 diseases have been so far reported on chickpea [4]. In general, soil borne diseases such as fusarium wilt (*Fusarium oxysporum f.sp. ciceris* Schlecht.), dry root rot [*Rhizocotonia bataticola* (Taub.) Butler], collar rot (*Sclerotium rolfsii* Sacc.) and black root rot [*Fusarium solani* (Mart.) Sacc.] are the major limiting factor in chickpea production in South Gujarat. Recently collar rot disease is emerging as a major threat to chickpea production.

Collar rot caused by *Sclerotium rolfsii* Sacc. is one of the devastating soil-borne disease of fungal origin, becoming more serious at seedling stage especially in the area where paddy or soybean based cropping system is followed [5]. However it's a serious threat to chickpea that may cause 55-95 per cent mortality of the crop at seedling stage under favorable environmental conditions [6].

Collar rot pathogen (*S. rolfsii*) could survive in the form of vegetative mycelium and/or sclerotia and causes rot of collar region on a wide range of plant species. Affected seedlings turn yellow and die. The seedlings generally collapse and show rotting at the collar region and below. Diagnostic signs of the fungus include characteristic white mycelial fans and brown sclerotia extending from infected tissues as well as soil. Collar rot is a fast spreading and destructive disease and is becoming more serious at seedling stage causing rot at collar region especially in area where paddy based cropping system is followed. As the genetic resistance is regarded, the only cost-effective control for such a devastating soil-borne pathogen is selection of cultivars. Therefore, the present study was carried out to evaluate the chickpea genotypes against *S. rolfsii* for the identification of resistant sources.

2. MATERIALS AND METHODS

2.1 Isolation of Pathogen and Inoculum Preparation

Pathogen was isolated from infected collar region of chickpea plant by directly transfer of sclerotia

and infected bits on potato dextrose agar (PDA) media under aseptic condition and plates were incubated at 27 ± 1^0C for optimum growth. The pathogen was purified by hyphal tip method and maintained on PDA slants. After purification, *Sclerotium rolfsii* was identified by observing the colony character and sclerotia formation. The morphological characters *viz.*, mycelial growth and mycelial characteristic were studied under high power magnification (40X) and sclerotia formation, shape and colour were studied under low power magnification (10X) from 10 days old culture of *S. rolfsii* and were compared with identification key described in "Illustrated Genera of Imperfect Fungi" [7]. Pathogenicity was proved on chickpea var. GG-5 by soil inoculation technique under pot conditions. Pathogen was multiplied on sorghum grains and 7 days old culture was used for inoculation.

2.2 Field Experiment

The experiment was conducted in randomized block design with thirty nine treatments and two replications during *Rabi* 2019-20 at Regional Rice Research Station, Navsari Agricultural University, Vyara. Thirty chickpea seeds were sown for each genotype at 30 x 10 cm distance in 3 m row length in field after inoculating with pathogen which was multiplied on sorghum grain. The inoculum was thoroughly broadcasted in soil @ 10g/ row. Germination and disease incidence [8] was recorded.

$$\text{Germination (\%)} = \frac{\text{No. of seeds germinated}}{\text{No. of total seeds sown}} \times 100$$

$$\text{Disease incidence (\%)} = \frac{\text{No. of diseased plant}}{\text{Total no. of observed plant}} \times 100$$

Collar rot of chickpea disease was assessed 10 days before harvesting as per 1-5 rating scale [9] described as 1= 0-10% plant mortality (resistant), 2= 11-20% plant mortality (moderately resistant), 3= 21-30% plant mortality (moderately susceptible), 4= 31-50% plant mortality (susceptible) and 5= 51-100% plant mortality (highly susceptible)

2.3 Statistical Analysis

Under field experiment simple RBD design with two replications was used. Germination percent and disease incidence data transformed in angular transformation for statistical analysis using OPSTAT software [10] by one way analysis of variance (ANOVA). The mean comparisons of genotypes were carried out by Duncan's Multiple Range Test where $P \leq 0.05$ was considered significant using OPSTAT software.

3. RESULTS AND DISCUSSION

The pathogen which was isolated from infected chickpea plants showed initially white fluffy mycelium appearance (Fig. 1a) and microscopic view of mycelium was hyaline, branching, compact with septate and had a clamp connection (Fig. 1b). White sclerotia were formed after 4^{th} days of incubation and later within 10 days after incubation it become mature and colour changed from brown to dark brown (Fig. 1c). Sclerotia appeared shiny due to presence of gummy material (Fig. 1d). All the above morphological characteristics of fungus were identified as *Sclerotium rolfsii* Sacc. and further confirmed with identification key described in "Illustrated Genera of Imperfect Fungi" [7]. In pathogenicity test cent per cent infection was observed in which pathogen caused infection first at collar region. Leaves of infected plant become pale green followed by yellowing. The profused white cottony growth of the fungus was observed near collar region of infected plant as well as in soil. Also similar morphological characteristics of mycelial growth and sclerotial formation was observed by earlier workers [11,12,13,14,15]. Pathogenicity was proved by sick soil method under earthen pot conditions and found that the pathogen in inoculated pot caused infection first at the collar region [16]. Leaves of such infected plants became pale green followed by yellowing. Similarly, pathogenecity of 10 isolates of *S. rolfsii* pathogen on groundnut by soil inoculation method and concluded that all the isolates of *S. rolfsii* infected the groundnut plant and the pathogenicity reactions ranged from 46.33 to 100 per cent [17]. The isolate S.r-9 exhibited maximum disease incidence (100%). Proved the pathogenicity of *S. rolfsii* causing collar rot in chickpea by soil inoculation method in pot condition and noticed that the pathogen caused infection on seedling resulting in mortality [18].

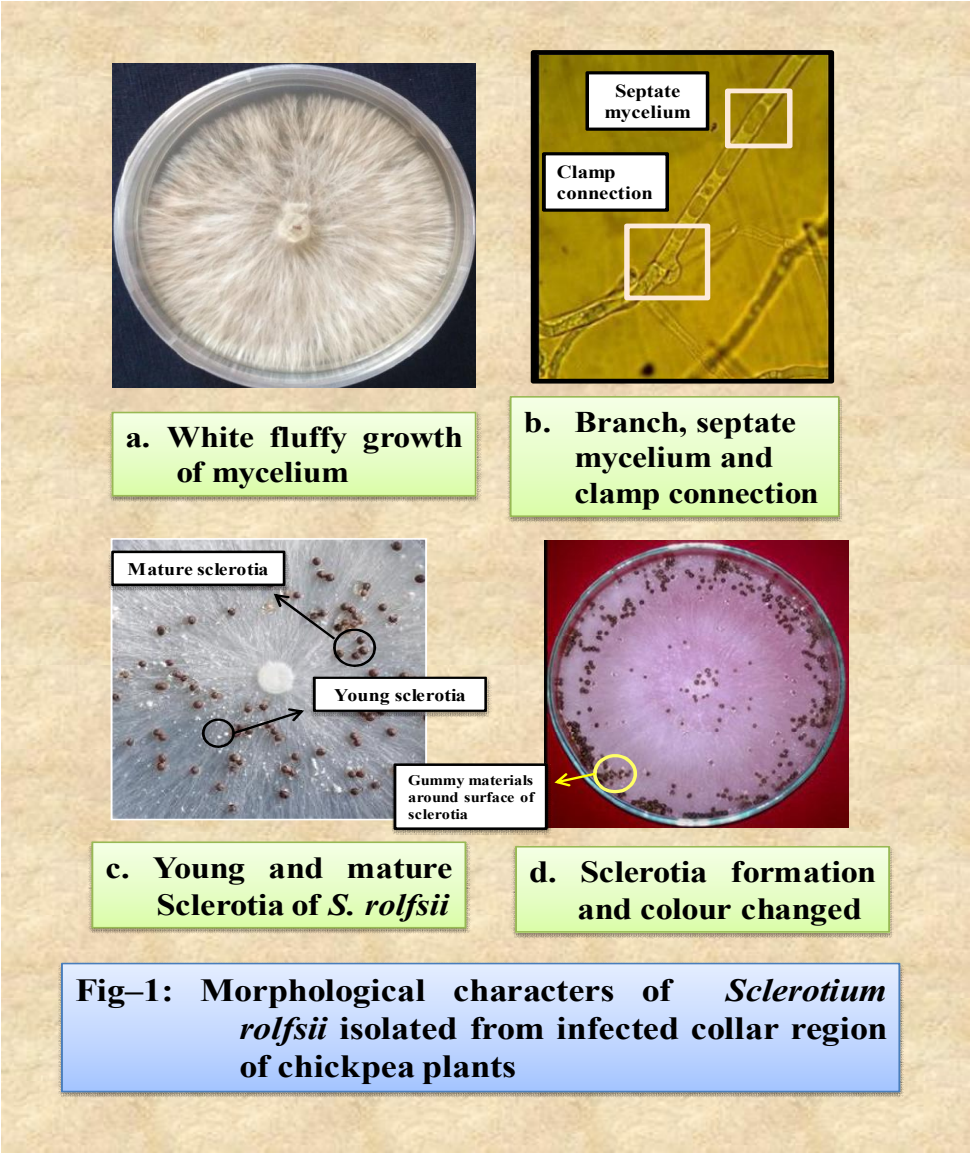
Table 1. Effect of *S. rolfsii* causing collar rot on seed germination and plant mortality of chickpea genotypes under field conditions

Sr. No.	Genotypes	Germination (%)*	Plant Mortality (%)*	Sr. No.	Genotypes	Germination (%)	Plant Mortality (%)
1	GG-1	47.14^{lmn}	24.26^{jklmnop}	21	GJG-1610	71.43^{abcdefghij}	29.22^{ghijklmno}
2	GG-2	48.57^{klmn}	23.21^{klmnop}	22	GJGK-1616	42.86^{n}	23.21^{klmnop}
3	GG-3	54.29^{hijklmn}	26.11^{ijklmnop}	23	GJGK-1617	60^{defghijklmn}	28.64^{ghijklmno}
4	GG-4	81.43^{abcd}	37.19^{efghijk}	24	GJGK-1618	61.43^{cdefghijklmn}	47^{bcdef}
5	GG-5	81.43^{abcd}	41.88^{bcdefgh}	25	GAG-1620	71.43^{abcdefghij}	39.61^{defghi}
6	GG-6	54.29^{hijklmn}	13.33^{p}	26	GJG-1707	57.14^{fghijklmn}	31.25^{ghijklmn}
7	BDG-72	72.86^{abcdefghi}	31.31^{ghijklmn}	27	GJG-1704	82.86^{abc}	55^{abc}
8	Chaffa	74.29^{abcdefgh}	46.22^{bcdef}	28	GJG-1707	65.71^{bcdefghijklm}	50^{bcde}
9	ICCC-2	70^{abcdefghijk}	38.97^{defghij}	29	GJG-1708	71.43^{abcdefghij}	34.13^{fghijklm}
10	Phule Vikaram	67.14^{bcdefghijkl}	17.41^{nop}	30	GJG-1710	55.71^{ghijklmn}	40.92^{cdefghi}
11	PKV-2	90^{a}	68.15^{a}	31	GJG-1712	71.43^{abcdefghij}	43.21^{bcdefg}
12	PKV-4	80^{abcde}	52.6^{bcd}	32	GJG-1713	44.29^{mn}	13.03^{p}
13	JGK-1	61.43^{cdefghijklmn}	18.33^{nop}	33	GJG-1714	50^{jklmn}	21.43^{lmnop}
14	Virat	78.57^{abcdef}	56.35^{ab}	34	GJG-1716	51.43^{ijklmn}	28.13^{hijklmno}
15	GJG-1503	85.71^{ab}	56.03^{ab}	35	GJG-1717	50^{jklmn}	28.33^{hijklmno}
16	GJG-1505	67.14^{bcdefghijkl}	29.71^{ghijklmno}	36	GJG-1720	54.29^{hijklmn}	23.33^{klmnop}
17	GJG-1509	55.71^{ghijklmn}	15.53^{op}	37	GNG-1722	77.14^{abcdefg}	55^{abc}
18	GJG-1511	58.57^{efghijklmn}	29.07^{ghijklmno}	38	GNaG-1723	54.29^{hijklmn}	26.67^{ijklmnop}
19	GJG-1603	64.29^{bcdefghijklmn}	35.57^{efghijkl}	39	Dahod yellow	72.86^{abcdefghi}	29.23^{ghijklmno}
20	GJG-1607	57.14^{fghijklmn}	20.2^{mnop}		*P value*	0.0001	0.0000
					C.V.%	14.30	17.92

*Mean of two replications *Original values*

Table 2. Reaction of chickpea genotypes against collar rot under field conditions

Rating Scale	Plant mortality (%)	Reaction	Genotypes
1	0-10	Resistant	Nil
2	11-20	Moderately Resistant	5- JG-1713, GG-6, GJG-1509, Phule Vikaram and JGK-1
3	21-30	Moderately susceptible	15- GJG-1607, GJG-1714, GG-2, GJGK-1616, GJG-1720, GG-1, GG-3, GNaG-1723, GJG-1716, GJG-1717, GJGK-1617, GJG-1511, GJG-1610, Dahod yellow and GJG-1505
4	31-50	Susceptible	13- GJG-1707, BDG-72, GJG-1708, GJG-1603, GG-4, ICCC-2, GAG-1620, GJG-1710, GG-5, GJG-1712, Chaffa, GJGK-1618 and GJG-1707
5	51-100	Highly Susceptible	6- PKV-4, GJG-1704, GNG-1722, GJG-1503, Virat and PKV-2



Fig–1: Morphological characters of *Sclerotium rolfsii* isolated from infected collar region of chickpea plants

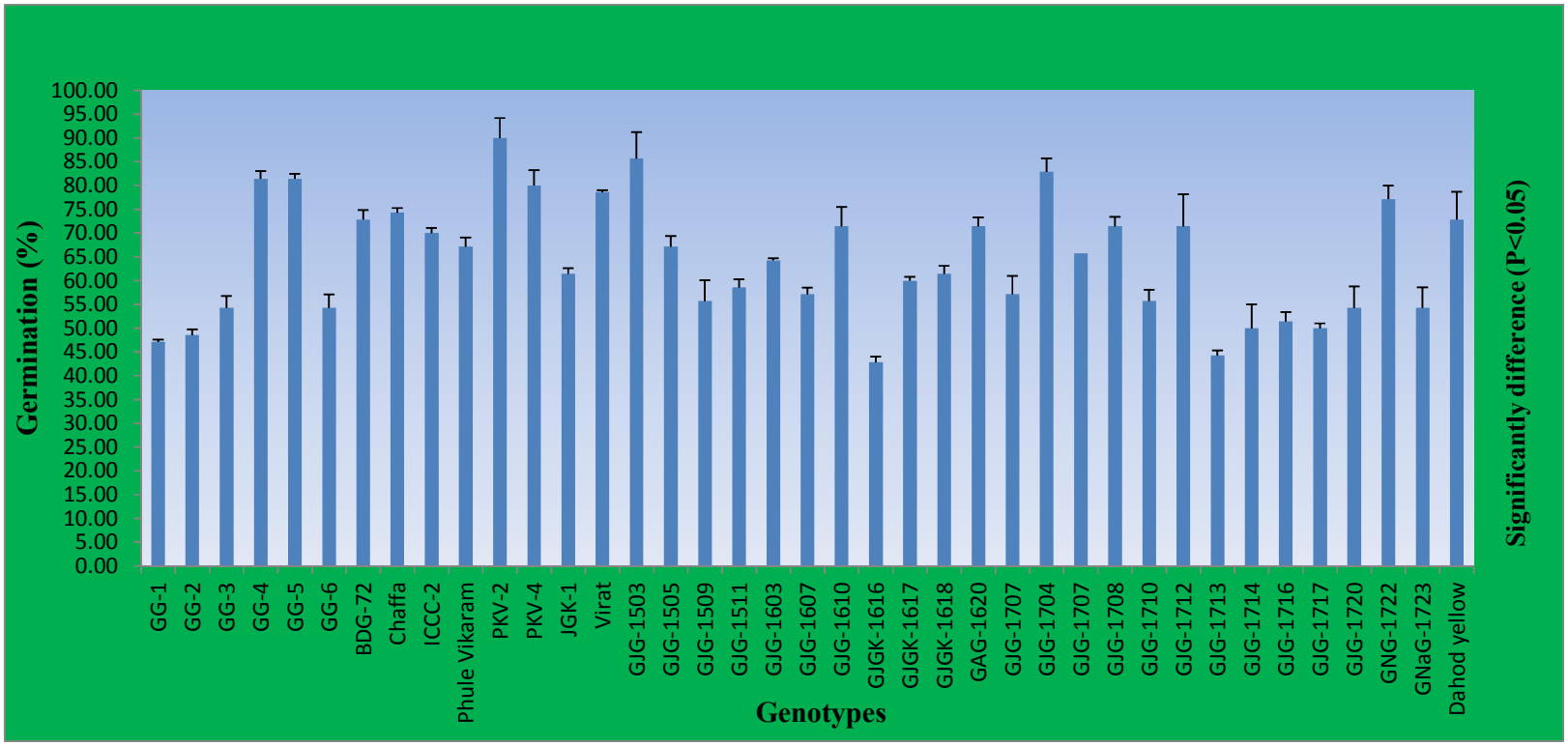


Fig. 2. Effect of *S. rolfsii* on seed germination of chickpea genotypes under field conditions

S.Em.± 6.52
C.D. at 5% 18.74
C.V. % 14.30
P value 0.0001

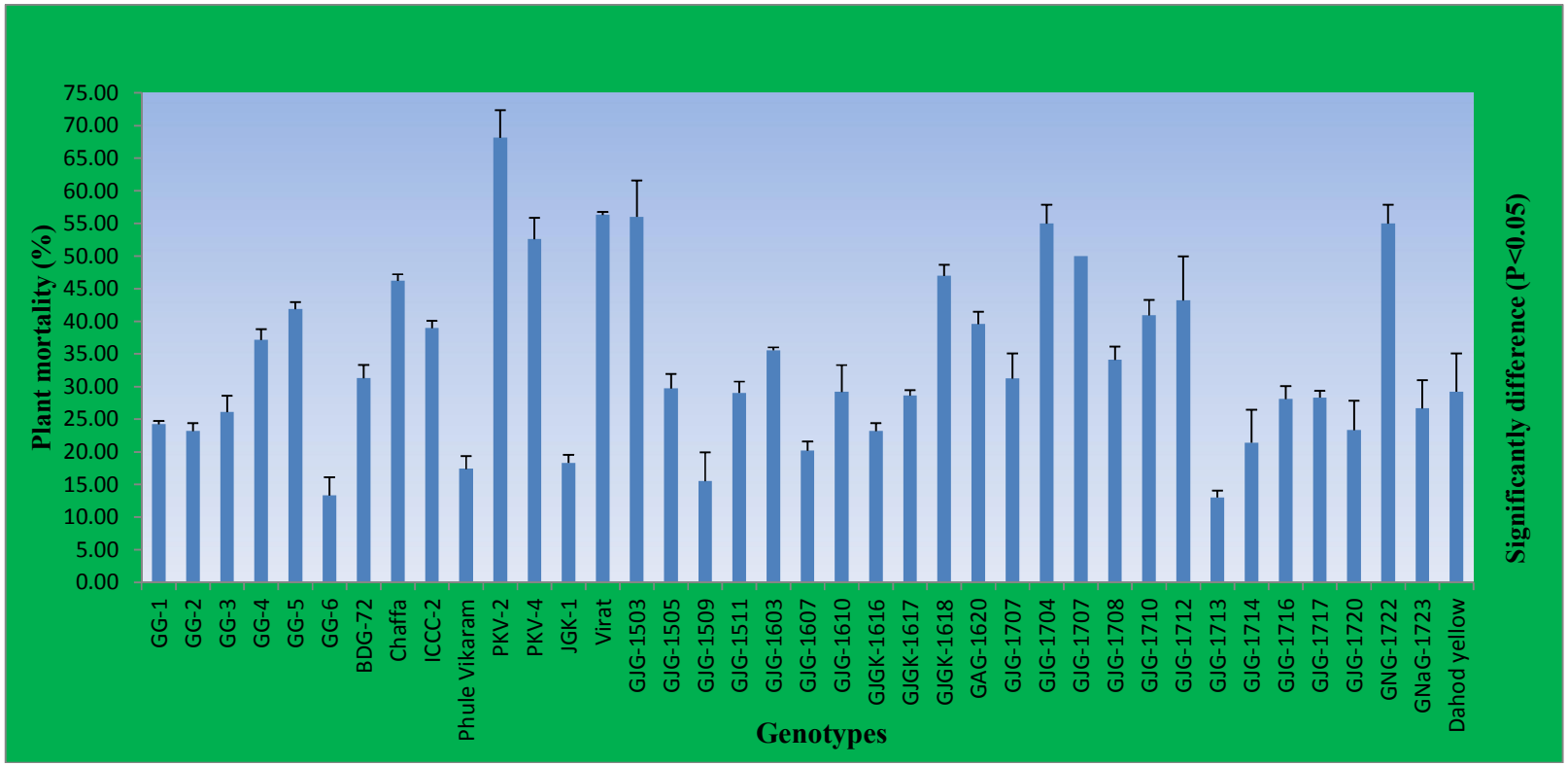


Fig. 3. Effect of *S. rolfsii* causing collar rot on plant mortality of chickpea genotypes under field conditions

S.Em.± 4.32
C.D. at 5% 12.41
C.V. % 17.92
P value 0.0000

Results of 39 chickpea genotypes was evaluated against collar rot disease caused by *S. rolfsii* under field conditions revealed that a significant difference (*P=0.05*) was observed between the genotypes for germination per cent (Fig. 2). Per cent germination was recorded in the range between 42.86 to 90.00 per cent in all the evaluated genotypes under field conditions (Table1). Highest germination was recorded in genotype PKV-2 with 90.00 percent which was at par with genotypes GJG-1503, GJG-1704, GG-4, GG-5, PKV-4, Virat, GNG-1722, Chaffa, BDG-72, Dahod Yellow, GJG-1610, GAG-1620, GJG-1708 and GJG-1712 with 85.71, 82.86, 81.43, 81.43, 80.00, 78.57, 77014, 74.29, 72.86, 72.86, 71.43 and 71.43 per cent, respectively. Among all the genotypes, the lowest germination was found in genotype GJGK-1616 with 42.86 per cent. Lowest seed germination may be due to pre-emergence rotting due to *S. rolfsii* under cold wet condition.

A significant variation (*P= 0.05*) was observed in plant mortality of different genotypes (Fig. 3). Per cent disease incidence as per cent plant mortality was observed in the range from 13.03 to 68.15 per cent in all the screened genotypes under field conditions (Table1). The lowest plant mortality (13.03%) was observed in genotype GJG-1713 which was at par with 10 genotypes *viz*., GG-6, GJG-1509, Phule Vikram, JGK-1, GJG-1607, GJG-1714, GG-2, GJGK-1616, GJG-1720 and GG-1 with 13.33, 15.53, 17.41, 18.33, 20.20, 21.23, 23.21, 23.21, 23.33 and 24.26 per cent plant mortality, respectively. Highest plant mortality (68.15%) was recorded in PKV-2genotype. The results of present experiment corroborated with earlier reports. They evaluated 36 chickpea germplams against *S. rolfsii* under sick soil condition and observed that out of these none of the germplasm was found resistant [19]. Screened out 284 chickpea germplasms [20] and they concluded that only 33 entries were found resistant, out of these 33 entries, 9 entries *viz*., IC 305641, IC 83515, IC117779, IC117783, IC117784, IC117792, IC117800, IC487500 and IC487394 were found free from disease infection and 24 entries showed <10 per cent plant mortality. Eighty four entries were exhibited 10.10 to 20.00 percent plant mortality and regarded as moderately resistant. Screened out total 206 chickpea entries (113 Kanpur desi, 61 Kabuli and 32 entries from ICRISAT) under field conditions against collar rot disease of chickpea and they found that among 206 chickpea entries, 136 entries were found resistant with 0.00-10.00 per cent disease incidence, only 44 entries showed moderate resistant with 11.00-20.00 percent disease incidence, whereas 16 entries showed moderately susceptible reaction with 21.00-30.00 percent disease incidence and 10 entries were found susceptible and highly susceptible with >31.00 per cent disease incidence [9]. Similarly, 185 chickpea entries screened out against collar rot disease caused by *S. rolfsii* under field conditions [21]. They found that only 5 entries *viz*., GNG 2331, JG 2016-9605, IPC 2012-98, RVSSG-38 and GL 12003 showed moderate resistant, whereas, 3 entries IPC 2013-33, NDG 14-24 and PG186 (ch) were identified as susceptible. None of the entries was found resistant against collar rot disease.

Out of 39 genotypes, none of the genotypes was found resistant. Only five genotypes *viz*., JG-1713, GG-6, GJG-1509, Phule Vikaram and JGK-1 showed moderate resistant reaction (table 2). While 15 genotypes viz., GJG-1607, GJG-1714, GG-2, GJGK-1616, GJG-1720, GG-1, GG-3, GNaG-1723, GJG-1716, GJG-1717, GJGK-1617, GJG-1511, GJG-1610, Dahod yellow and GJG-1505 exhibited moderate susceptible reaction and 13 genotypes showed susceptible reaction. Six genotypes like PKV-4, GJG-1704, GNG-1722, GJG-1503, Virat and PKV-2 showed highly resistant reaction against collar rot disease.

4. CONCLUSION

S.rolfsii fungus was most prevalent pathogen caused collar rot disease in chickpea under rice based cropping system of South Gujarat. Out of 39 chickpea genotypes, only five genotypes *viz*., GJG-1713, GG-6, GJG-1509, Phule Vikram and JGK-1 were showed moderate resistant reaction collar rot disease. Whereas, 15 genotypes showed moderate susceptible reaction, 13 genotypes showed susceptible reaction and 6 genotypes showed highly susceptible reaction. None of the genotypes were found resistant against collar rot disease under inoculums inoculated rice fellow cropping system. Moreover, genotypes GJG-1713, GG-6, GJG-1509, Phule Vikram and JGK-1 can be used in the breeding program for development of resistant variety for the management of collar rot disease.

DATA AVAILABILITY STATEMENT

The original data presented in the study are included in the article and supplementary

material; further inquiries can be directed to the corresponding author's.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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