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Isolation, identification, pathogenicity and screening of clusterbean varieties/germplasm against wilt of clusterbean

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Abstract

The clusterbean [*Cyamopsis tetragonoloba* (L.) Taub.] is belong to the *Fabaceae* family that derived from the Sanskrit word "Gauaahar," which means "livestock fodder. Wilt of clusterbean caused by *Fusarium solani* is a severe problem in clusterbean growing regions, the severity of the disease varies by region and it creates severe plant loss at the pre-emergence and post-emergence stages. Wilt of clusterbean caused by *Fusarium solani* is a major disease that causes severe seed yield losses among all fungal, bacterial and viral diseases. The pathogen was isolated from diseased root portions and purified using hyphal tip cut techniques before being identified as *Fusarium solani* based on morphology and colony characteristics. Pathogenicity was also shown under pot conditions using Koch's postulates procedures which also included seed, soil inoculation and seed cum soil inoculation. Seed cum soil inoculation method found most effective followed by soil inoculation method. The disease's first symptom appeared on the 10th day after sowing and characteristic wilt symptoms such as yellowing plants, drooping leaves and browning and blackening of vascular tissues appeared within 30 days of sowing. The pathogen was re-isolated and the results were compared to the original cultures.

To identify variation among different collected isolates various isolates were isolated from samples collected from different clusterbean growing studied areas of Rajasthan and categorized based on their morphological and cultural characteristics. Colony diameter, colony development characteristics, sporulation rate and conidial morphology significant differences among *F. solani* isolates. Cottony white mycelium with brown pigmentation was found in the majority of the isolates. The number of septations in macro-conidia ranged from 2-3 to 0-1 in micro-conidia. Twelve clusterbean varieties/genotypes were tested against *Fusarium solani* under field conditions during *kharif* 2019 and *kharif* 2020. RGC-1038, RGC-1002, RGC-1003 and RGr12-1 were found to moderately resistant. RGC-1017, RGC-1033, RGC-936, RGC-1066, RGC-986 and RGC-1031 were found to susceptible to clusterbean wilt whereas RGC-1055 and RGC-197 were found to be highly susceptible.

Keywords: Clusterbean, *Fusarium solani*, Pathogenicity, Genotypes

Introduction

Clusterbean [*Cyamopsis tetragonoloba* (L.) Taub.] is an important arid legume crop. It is grown for many different purposes *viz*., vegetable (pods), green fodder, straw, green manure, guar gum, seed production, cattle fodder and increase soil fertility (Yogi *et al*., 2016) [20]. Clusterbean is a popular annual legume crop with primary source of guar gum. It is mostly grown on upland sandy loam and loam soils which are well drained. It needs a temperature range of 30 to 35°C to grow and flourish. During the vegetative stage, guar plants are ploughed down and mixed with the soil to promote soil fertility. They absorb a significant quantity of nitrogen from the atmosphere by adding organic matter (Rewari *et al*., 1957) [14]. It is cultivated in India for the production of seeds and the extraction of guar gum. The endosperm of its seeds contains 18% protein, 32% fibre and approximately 30-33% gum. The seed coat (14-17%), endosperm (35-42%) and germ (43-47%) represent the three components of the guar seed. Guar has become a major industrial cash crop due to the existence of glactomannan gum and it is the primary marketable product derived from guar endosperm (Sharma and Gummagolmath, 2012) [16]. Guar gum trading in India accounted almost for 80% of global trade and guar seed was one of the top three traded agricultural commodities on Indian exchanges (Mishra, 2008) [10].

The crop suffers from vagaries diseases *viz*., bacterial blight (*Xanthomonas axonopodis* pv. *cyamopsidis*), Alternaria blight (*Alternaria cyamopsidis*), Anthracnose (*Colletotrichum capsici* f.sp. *cyamopsicola*), *Curvularia* leaf spot (*Curvularia lunata*), charcoal rot/damping off (*Macrophomina phaseolina*), wilt (*Fusarium solani*), Myrothecium leaf spot (*Myrothecium roridum*) and powdery mildew (*Oidiopsis taurica*). Among these important and fatal diseases, wilt caused by *Fusarium solani* (Mart.) Sacc. is one of the most important disease during the current era. In India, this disease was first identified and reported by Singh (1951) [17] from Kanpur and Vir and Grewal (1973) [18] from Delhi. In Rajasthan Mathur and Shekhawat (1988) first time reported *Fusarium solani* on guar (*Cyamopsis tetragonaloba*) from Durgapura, Rajasthan. It is commonly grown in India, Pakistan, the United States, Morocco, Italy, Germany, Greece and Spain (Hymowitz and Matlock, 1963) [5]. In India, the total area under guar production is around 4.2 million ha with 1.89 million tonnes of guar seed production (Anon., 2017-18) [2]. Rajasthan contributes about 70% of India's total production. It is also grown in Haryana, Gujarat, Punjab, Uttar Pradesh, Rajasthan and Madhya Pradesh (Pandey and Roy, 2011) [11]. The total area, production and productivity of the clusterbean in India is 3.14 million hectares, 1.52 million tones and 484.0 kg/ha, respectively (Anonymous, 2021a) [1]. In Rajasthan, the major clusterbean growing districts are Barmer, Bharatpur, Bhilwara, Bikaner, Churu, Jalore, Jodhpur, Nagaur, Jaisalmer, Jhunjhunu, Sikar, Jaipur and Sriganganagar. The total area and production in Rajasthan is 2.5 million hectares, 1.1 million tonnes, respectively and productivity was 458 kg/ha. The productivity of clusterbean in Rajasthan is 452 kg/ ha which is far below its potential yield, *i.e*., 1350 kg/ ha (Anon., 2021b) [1].

Materials and Methods

Isolation and purification of pathogen

Isolation of pathogen was made on PDA medium from individual wilt infected root of the plant collected from field showing maximum disease incidence during survey. The samples of diseased plants were used for isolation of the causal fungus. The affected parts were thoroughly washed with tap water to remove soil. Small pieces of about 0.5 cm length were cut from roots and surface sterilized with 1 per cent sodium hypochlorite solution for 1 minute followed by three washings with sterilized distilled water. The surface sterilized pieces were transferred aseptically on Potato Dextrose Agar (PDA) slants in a Laminar Air Flow Cabinet and incubated at 25±1 °C temperature in a B.O.D. incubator for 7 days. To obtain pure culture of *Fusarium solani*, single hyphal tip isolation technique was adopted (Rangaswami and Mahadevan, 2004). Single piece of hypha was demarcated under low power objective of microscope (10×) and cut with the help of dummy objective. Individual piece of hypha was transferred aseptically on PDA slants with the help of an inoculating needle. The inoculated slants were kept in B.O.D. incubator at 25±1 °C temperature. Purified cultures of eight samples were tentatively identified as *Fusarium solani* on the basis of morphological and colony characters. The purified cultures were maintained by periodical transfers on PDA slants and used for further studies. One representative isolate from each surveyed district was established from the field showing maximum disease incidence.

Identification of the pathogen

The isolated fungus was identified on the basis of morphological and colony characters *viz.,* cottony white aerial mycelium instead developing a pink or violet centre. The fungus produces micro conidia, macro conidia and chlamydospores. Micro conidia are ovoid and formed terminally on elongated micro conidiophores. Macro conidia are sickle shaped 2 to 4 septate, slightly curved, rounded at both ends, thick walled and produce abundently in 10-15 days old culture. Chlamydospores are smooth, globose, measuring 9.2x7.5 µm and formed solitary or in chains.

Pathogenicity test

For proving pathogenicity, isolated and purified *Fusarium solani* was multiplied on sterilized sorghum grains and proved pathogenicity through seed, soil and seed cum soil inoculation techniques. Soil collected from the field was autoclaved at 1.05 kg/cm^2 pressure for 30 minutes for three consecutive days. The inoculum multiplied on sorghum grains was added in the earthen pots (30 cm diameter) @ rate of 20 g/pot. The pots were surface sterilized by 2 per cent formalin solution before filling the soil inoculum mixture. Ten apparently healthy clusterbean seeds of susceptible variety RGC-1055 were surface sterilized with 1 per cent sodium hypochlorite solution were sown in each pot with four replications. The pots without inoculum were served as control. Observations for disease incidence was recorded 40 days after sowing (DAS). Plants showing wilt symptoms were collected and the pathogen was re-isolated and compared with the original culture (Radha Krishanan and Sen, 1985).

Seed inoculation technique

For this, seeds were rolled on 7 days old culture of fungus thiriving on PDA contained in Petri plates. The inoculated seeds were sown in cemented pots. The un–inoculated apparently healthy seeds served as control. These pots were kept in cage house and watered regularly as and when required.

Soil inoculation technique

Prior to sowing, pots (30 cm diameter) were sterilized with formalin solution and filled with sterilized soil + FYM (Soil: FYM=3:1; sterilized at 1.045 kg/cm^2 for one hour for three consecutive days). These pots were inoculated with inoculum, multiplied on sorghum grains @ 20 g/pot. Healthy and surface sterilized ten clusterbean seeds (RGC-1055) were sown in each pot with four replications. Surface sterilized seeds sown in uninoculated sterilized soil, served as control. These pots were kept in cage house and watered regularly as and when required and maintained under identical conditions.

Seed cum soil inoculation technique

In this technique above both techniques were carried out together and inoculum of pathogen was applied through soil and seed with 7 days old culture of fungus and surface sterilized seeds sown in uninoculated sterilized soil, served as check.

Effect of varietal screening on wilt incidence and seed yield of clusterbean

Twelve varieties/genotypes of clusterbean *viz.,* RGC-1055, RGC-1017, RGC-1033, RGC-936, RGC-197, RGC-1066, RGC-1038, RGC-986, RGR 12-1, RGC-1002, RGC-1003 and RGC-1031 were collected from RARI Durgapura for evaluating against wilt during *Kharif* 2019 and 2020. Inoculum multiplied on sterilized sorghum grains was added in furrows @ 20 g/m row length. Three replications were maintained for each variety. Observations on disease incidence were recorded 40 days after sowing. On the basis of disease incidence, the varieties were categorized as per criterion followed by Iqbal *et al*. (2005) [6] and Farooq *et al.* (2019) [4].

Category	Per cent disease incidence
Highly resistant	0-10%
Resistant	11-20%
Moderately resistance	21-30%
Susceptible	31- 50%
Highly Susceptible	More than 50%

Results and Discussion

Isolation and identification of pathogen: Plants showing typical symptoms of wilt disease were collected during survey from every surveyed field of four districts *viz*., Jodhpur, Sikar, Jaipur and Nagaur districts and brought to the laboratory. On the basis of maximum disease incidence, one sample was chosen among eight isolates of eight tehsil for isolating pathogen and establishing a representative isolate of a tehsil. Thus, selected samples of diseased plants were used for isolating causal fungus under aseptic conditions by incubating in BOD at 25+1 °C in Petri plates containing Potato Dextrose Agar (PDA) medium. Pure culture of the pathogen was obtained by hyphal tip technique. Thus, eight isolates of the pathogen was established and coded.

The fungus develops sparse to abundant, white creamy mycelium that eventually turns pinkish in colour. The hyphae were septate and hyaline more or less pink in colour. The colonies were pinkish white coloured and reverse usually colourless to light pink. All of the isolates cultures produced a number of macro and microconidia. Microconidia borne on short conidiophores are many elliptical, round to oval in shape and have 1-2 septa. Macroconidia are 3 - 4 septate, falcate fusoid, sickle-shaped or slightly curved with a blunted apical end (Mehrotra and Aneja, 2015) [9]. On the basis of these cultural and morphological characteristics of the fungus all pure cultures of different isolates were classified as *Fusarium solani* and compared to the standard description (Barnet and Hunter, 2003; Leslie and Summerell, 2006) [3, 7].

The important symptoms of the disease were plants gradually turned into yellow, leaves drooped and, typical browning and blackening of vascular tissues. The symptoms observed during the present survey and also noticed in the research experiments are in accordance with observations of the earlier scientists (Pareek and Varma, 2014, Saini and Goyal, 2016) [12, 15]. Saini and Goyal (2016) [15] first reported *Fusarium pallidoroseum* which causes wilt disease in clusterbean.

Pathogenicity of *Fusarium solani*

The pathogenicity of isolated and purified *Fusarium solani* was tested under pot house conditions on clusterbean variety RGC-1055 by seed, soil and seed + soil inoculation techniques. The fungus was found highly pathogenic to cause wilt of clusterbean. After 30-40 days after sowing typical wilt disease symptoms was observed. The infected plants showed regular wilting symptoms such as plants becoming yellow and leaves drooping. Later typical browning and blackening of vascular tissues also found.

The symptoms observed in artificially inoculated plants were similar to those visualized in naturally infected plants during survey. Re-isolation from the infected region of inoculated and diseased plants further yielded the fungus identical to one used for artificial inoculation of the plants. The un-inoculated plants were free from any type diseased symptoms.

Effect of varietal screening on wilt incidence of clusterbean

Twelve varieties of clusterbean were screened during *Kharif* 2019 and 2020 in artificially soil inoculated field conditions against wilt disease. The observations on disease incidence on various varieties were recorded and categorized as per their reaction.

The results of pooled data revealed that none of clusterbean variety was found immune, resistant and moderately resistant. RGC-1038, RGr 12-1, RGC-1002 and RGC-1003 were found moderately susceptible as these showed 29.15, 24.82, 28.83 and 23.34 per cent disease incidence, respectively. Further, six varieties namely RGC-1017 (38.22% incidence), RGC-1033 (31.73% incidence), RGC-936 (36.36% incidence), RGC-1066 (42.19% incidence), RGC-986 (33.92% incidence) and RGC-1031 (43.63% incidence) were found susceptible whereas two varieties namely RGC-1055 (58.78% incidence) and RGC-197 (51.16% incidence) were found highly susceptible to the disease. In general, testing of resistance is a continuous process because of evolution of new biotypes of the pathogen or it may be due to break down of resistance in host genotypes. Plant diseases management with the principal of host resistance is an economic and environmentally safe. In the present case, out of twelve varieties of clusterbean, tested for two consecutive cropping seasons, that none of clusterbean variety was found immune, resistant and moderately resistant but four varities *viz*., RGC-1038, RGr 12-1, RGC-1002 and RGC-1003 were found moderately susceptible. Similar Yadav *et al*. (2020) [19] screened ten varieties of clusterbean against *Fusarium solani* under artificial inoculation condition. They found none of the variety show resistant reaction *Fusarium* wilt and RGC-197 was found susceptible. According to Patel *et al.* (2002) [13] genotypes of guar *viz.*, GAUG 998, GAUG 9112, GAUG 605 and GAUG 9010 showed moderate resistance to wilt (*Neocosmospora vasinfecta*).

Table 1: Screening of different varieties/ germplasm lines of clusterbean against wilt disease

S. No.	Varieties	Per cent disease incidence 2019	Per cent disease incidence 2020	Pooled	Disease reaction
1	RGC-1055	57.15	60.40	58.78	HS
		(49.11)	(51.00)	(50.05)	
2	RGC-1017	35.30	41.13	38.22	S
		(36.45)	(39.89)	(38.18)	
3	RGC-1033	30.20	33.25	31.73	S
		(33.34)	(35.21)	(34.28)	
4	RGC-936	34.30	38.41	36.36	S
		(35.85)	(38.30)	(37.08)	

5	RGC-197	49.12	53.20	51.16	HS
		(44.50)	(46.83)	(45.66)	
6	RGC-1066	40.18	44.20	42.19	S
		(39.34)	(41.67)	(40.51)	
7	RGC-1038	27.40	30.90	29.15	MR
		(31.56)	(33.77)	(32.68)	
8	RGC-986	31.50	36.33	33.92	S
		(34.14)	(37.07)	(35.62)	
9	RGr12-1	23.14	26.51	24.82	MR
		(28.75)	(30.99)	(29.88)	
10	RGC-1002	27.2	30.45	28.825	MR
		(31.44)	(33.49)	(32.47)	
11	RGC-1003	21.15	25.53	23.34	MR
		(27.38)	(30.35)	(28.89)	
12	RGC-1031	42.15	45.11	43.63	S
		(40.48)	(42.19)	(41.34)	
	S.Em+	1.55	1.75	1.88	
	CD (P=0.05)	4.78	5.39	5.79	
	CV	8.35	8.47	8.10	

* Average of three replications
Parenthesis are angular transformed value

References

1. Anonymous. Directorate of Economics and Statistics, Department of Agriculture and Cooperation, Ministry of Agriculture & Farmers Welfare, Govt. of India, New Delhi; c2021a.
2. Anonymous. DOA, Department of Agriculture, Govt. of Rajasthan, Jaipur, www.krishi.rajasthan.gov.in. 2022b.
3. Burnett HL, Hunter BB. Illustrated genera of imperfect fungi. 4th edition, Burgess Publishing Company. Minneapolis, Minnesota, USA. 2003.
4. Farooq S, Mohyo-Ud-Din A, Naz S, Siddique M, Khan SN. Screening of sesame (*Sesamum Indicum* L.) germplasms for resistance against charcoal rot disease caused by *Macrophomina Phaseolina* (Tassi) Goid. I J Bio and biotech. 2019;16(2):407-410.
5. Hymowitz T, Matlock RS. Guar in the United States. Okla Aes Bull B6. 1963;11:1-34.
6. Iqbal SM, Haq IU, Bukhari A, Ghafoor A, Haqqani AM Screening of chickpea genotypes for resistance against *Fusarium* wilt. Mycopathology. 2005;3:1-5.
7. Leslie JF, Summerell BA. The *Fusarium* Laboratory manual. (Blackwell Publishing: Iowa, USA). 2006.
8. Mathur K, Shekhawat KS. *Fusarium* root rot of guar. Indian Journal of Mycology and Plant Pathology 1988;17:237.
9. Mehrotra RS, Aneja KR. An introduction to mycology. New Age International Private Limited, New Delhi. 2015;714.
10. Mishra S. Indian guar gum exports up on industry demand. Reuters, April 28, 2008.
11. Pandey KC, Roy AK. Forage crops varieties. Indian Grassland and Fodder Research Institute, Jhansi, Uttar Pradesh, India; c2008. p. 66-68.
12. Pareek V, Varma R. Phytopathological effects and disease transmission in clusterbean seeds grown in Rajasthan. Indian Journal of Plant Science. 2014;3(2):26-30.
13. Patel DS, Patel SI, Desai AG. Clusterbean varietal to wilt disease caused by *Neocosmospora vasinfecta* E.F. Smith. Journal of Mycology and Plant Pathology. 2002;32:120-21.
14. Rewari RB, Sen A, Pandey SL. Excretion of nitrogen from the roots of guar bean. Journal of Indian Society of Soil Science. 1957;5(4):237-245.
15. Saini S, Goyal M. First report of *Fusarium pallidoroseum* (Cooke) Sacc. causing wilt disease of *Cyamopsis tetragonoloba* (Clusterbean) in Rajasthan. Indo American Journal of Pharmaceutical Research. 2016;6(4):5070-5073.
16. Sharma P, Gummagolmath KC. Reforming Guar Industry in India: Issues and Strategies. Agric Econ Res Rev. 2012;25(1):37-48.
17. Singh RS. Root rot of guar. Sci Cult. 1951;17:131-134.
18. Vir S, Grewal JS. Studies on the physiology of *Fusarium caeruleum* the causal organism of guar wilt. Indian Phytopathology. 1973;26:72-75.
19. Yadav P, Ahir RR, Babu R, Saini DK, Deora A, Sumitra. Survey and host plant resistance of cultivars in clusterbean against *Fusarium* wilt caused by *Fusarium solani*. International Journal of Current Microbiology and Applied Science. 2020;9(8):3680-3685.
20. Yogi R, Sharma OP, Verma HP, Kanwar K, Shivran AC. Response of fertility and agro-chemical on growth and yield of different varieties of clusterbean. Annals Agriculture Research New Series. 2016;s37(3):295-299.