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Comparative Assessment of Inoculation Techniques for Development of Dry Root Rot Disease in Chickpea (*Cicer arietinum* L.)

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Abstract
Chickpea is important legume crop in India and contribute 64 percent of the world's chickpea production. Drought, heat and dry root rot are major biotic and abiotic factors to limit chickpea production. Currently soil borne pathogen, *Rhizoctonia bataticola* causes dry root rot and more yield reduction in chickpea during flowering to maturity stage. An investigation was conducted in chickpea (*Cicer arietinum* L.) genotype, JG-62 at Mahatma Phule Krishi Vidyapeeth, Rahuri during the year 2020-21. Four methods of screening viz. paper towel method, seed inoculation method, pot inoculation method and drenching of mycelium suspension method for dry root rot disease were standardized in green house. Mycelium suspension drenching method exhibited maximum disease incidence which was followed by paper towel, pot inoculation and seed inoculation method. Minimum disease incidence observed in seed inoculation method. This results will help the researchers to select the screening technique to screen chickpea genotype for dry root rot disease.

Keywords: Chickpea, dry root rot, screening technique

Introduction
Legumes comprises grain, pasture and agro-forestry species from which grain and forage legumes contribute 27% of the world's primary crop production [1]. Grain legumes contribute significant part to vegetarian diet as they are good source of calcium, protein, iron, phosphorus, minerals and protein content which is more than other food items [2]. Chickpea is considered as important legume crop which is an excellent source of protein, minerals and contributes 30% of total pulses production of the world [3]. Under field conditions, chickpeas are vulnerable to a number of fungal diseases which severely damaged to the crop and negatively impact on the national economy [4]. Serious diseases in chickpea as reported are Ascochyta blight, wilt, wet root rot and dry root rot which shold be controlled to solve the problem of malnutrtion [4].

Dry root rot (DRR) is potentially devasting disease which is caused by *Rhizoctonia bataticola* (Taub.) or *Macrophomina phaseolina* (Tassi), more prevalent in chickpea farms in india and cause 10 to 20 % annual loss [5,6]. This pathogen produces black microsclerotia and spreads through dark-branched hyphae when it interacts with chickpea. DRR in chickpea, a soil-inhabiting, seed and soil borne fungus which caused rotting destruction of lateral root and plugging of xylem vessels [7]. For identification of resistant genotype for particular disease, there is need to screen the genotype [8]. There are several methods available to screen germplasm viz. sick plot method, paper towel method and pot culture method for identification of resistant sources which are studied by several researchers. Screening methods vary in terms of their results. The chances of identification of resistant source for disease is increased as more reliable screening methods used.

The assessment of different methods require evaluation of methods in green house as well as in vitro condition. So, there is need to identify and standardize different screening methods for evaluation of response of different genotype to dry root rot. Using different methods, we can characterize the different germplasm at a time for particular disease. There is need to evaluate the comparative performance of screening techniques which can identify resistant sources in less time. So, the current study was
conducted to determine the performance of various screening techniques against dry root rot disease of chickpea.

1. Materials and Methods

The present study was conducted at Mahatma Phule Krishi Vidyapeeth, Rahuri during the year 2020-21. Four different inoculation methods *viz.* paper towel method (Nene *et al*., 1981), seed inoculation method [9], pot (soil) inoculation method [10] and drenching of mycelium suspension method [11] were evaluated to find out best strategy for screening dry root rot disease on JG-62 chickpea genotype.

2.1 Collection and isolation of the fungus

The chickpea plants showing typical symptoms of dry root rot were collected from experimental plots of chickpea at MPKV, Rahuri (Plate 1a and 1b) and the samples were brought to the laboratory and washed under running tap water and blot dried. Infected roots were cut into small bits of size 5-6 mm, surface sterilized by 0.2 % mercuric chloride solution or dipping in 0.8% sodium hypochlorite for 2 min. After thorough washing repeatedly thrice in sterile distilled water, pieces of roots were transferred to sterile potato dextrose agar medium in petri dishes and incubated at 27 ± 1°C to obtain mycelial growth. The 5mm grown fungus in plate from 7-day-old fully developed pure culture were transferred into test tube slants containing potato dextrose agar media in order to preserve the natural strains of *R. bataticola*. The test tubes were then incubated in a dark environment at 27 ± 2 °C for 7 days until the pathogen had grown completely in the test tubes [12]. After fungal growth, different techniques for screening of dry root rot disease were standardized.

Plate 1: Photograph depicting symptoms of dry root rot infected plants

2.2 Blotter paper technique

The protocol used for screening chickpea genotypes in the laboratory condition was as follows (Plate 2);

Screening of chickpea genotype against dry root rot through blotter paper technique (*in vitro*) was employed [13]. Initial seeds of JG-62 genotype surface sterilized and sown in germination chamber in three replications (Plate 2a). Potato Dextrose Broth (PDB) was prepared to culture the fungus *Rhizoctonia bataticola* and flasks were inoculated with the fungus and incubated at 35°C for seven days (Plate 2b). The mycelial mats were removed from the flask which were added to 100 ml sterilized distilled water in a beaker after its proper crushing for 1 minute in the blender. Seven days old seedlings were dipped in the fungus inoculum and kept in a beaker for about 30 seconds (plate 2c). Ten seedlings of the test line were taken and 10 of these were kept separately on blotter papers (size 45 cm x 25 cm with one fold). The folded blotter papers were kept one on top of the other in a tray. These trays were placed into the incubator at 35 °C for 8 days which was provided with artificial light

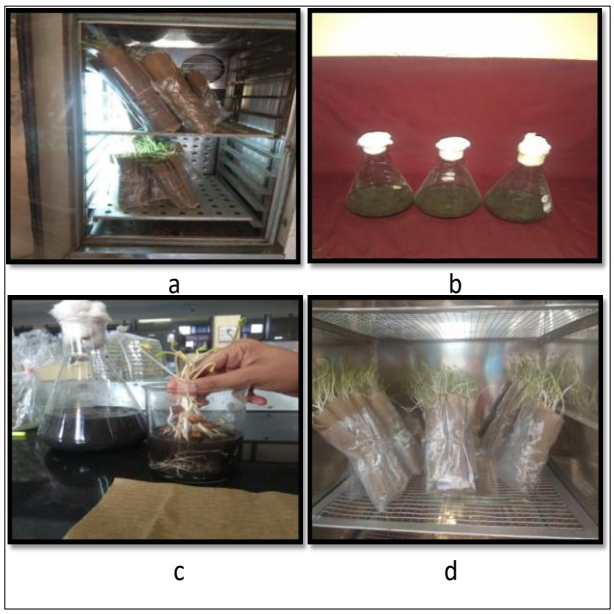

Plate 2: Blotter paper technique

for 12 hrs. Disease was scored on 1-9 point scale (Table 1) for *in vitro* condition [13] (Plate 2d).

Table 1: Disease scoring scale (*in vitro* condition) for dry root rot disease

Rating	Category	Symptoms of DRR
1	Resistant	No infection on roots
2-3	Moderately resistant	Very few small lesions on roots
4-5	Tolerant	Lesions on roots clear but small; new roots free from infection
6-7	Susceptible	Lesions on roots many; new roots generally free from lesions
8-9	Highly susceptible	Roots infected and completely discolored

2.3 Pot (soil) Inoculation technique

The fungus cultured on petri-plate was multiplied on sand: sorghum medium (7:3) by inoculating in bags which was autoclaved and incubated at 28±2 °C for 15 days (Plate 3a and 3b). Further the *R. bataticola* inoculum added to the sterilized soil @ 50 gm/kg soil. The pots were kept for 15 days for stabilization before sowing. Each treatment were maintained in three replication. Ten seed of JG-62 were sown in sick pot as well as in controlled conditions. Observations of disease incidence were recorded at weekly interval using disease scale (Table 2).

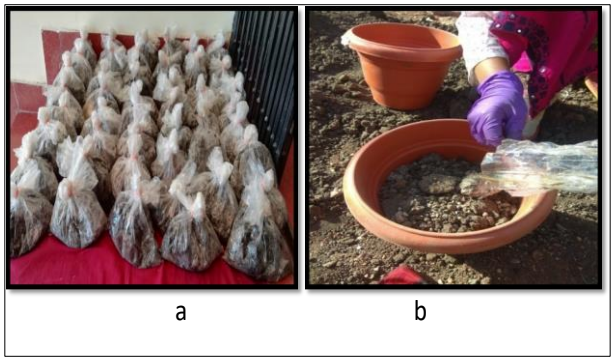

Plate 3: Pot (soil) Inoculation technique

2.4 Seed inoculation technique

Ten healthy surface sterilized seeds of genotype JG-62 were inoculated with culture of *Rhizoctonia bataticola* fungus thriving on PDA in petri-dishes (Plate 4a). Then inoculated seeds were plated on moist blotter papers in petri dishes. Ten seeds were plated at nearly equal distance in each petri-dish. After plating, petri-dishes were incubated. After incubation, seeds of JG-62 were sown in pots in three replications (Plate 4b) and observations were recorded at weekly intervals and disease incidence was calculated (Table 2). Seeds of JG-62 was also sown in controlled condition, separately.

Plate 4: Seed inoculation technique

2.5 Drenching of mycelium suspension technique

Ten seeds of genotype JG-62 were sown in pot in three replications and fourteen days old seedlings were treated with mycelium suspension. Potato dextrose broth was prepared and 100 ml of broth was placed in 250 ml flasks. Thereafter broth was autoclaved and inoculated with fungus. Further the mycelial mats were removed from flasks at the end of the incubation period. Mycelial mats were blended in a mixer with 100 ml sterilized distilled water and drenched in each pot (Plate 5a and 5b). Seeds of JG-62 lines were sown in controlled condition separately. Observations were taken after one week of treatment and disease incidence was calculated (Table 2).

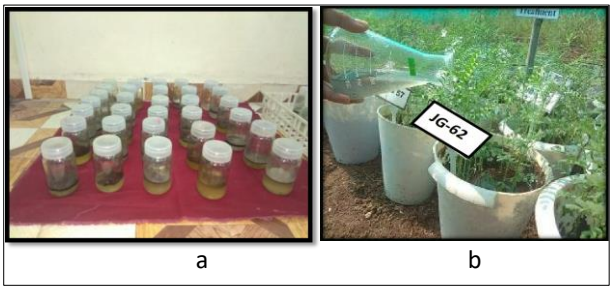

Plate 5: Drenching of mycelium suspension technique

Table 2: Rating scale used for disease scoring in field

Sr. No.	Category	DRR %
1.	Resistant	0.0-10
2.	Moderately resistant	10.1-20
3.	Moderately susceptible	20.1-30
4.	Susceptible	31-50
5.	Highly susceptible	50 and above

The reaction of genotype as disease incidence was calculated by the following formula.

$$\text{Incidence (\%)} = \frac{\text{No. of diseased plants}}{\text{Total plants}} \times 100$$

2.6 Statistical analysis

Statistical analysis was done for the interpretation of the results obtained. The experiments were laid out in CRD and analysis was done accordingly.

3. Result and Discussion

The fuzzy aerial mycelium of the fungus observed in the petri plate ranged in color from grey to black (plate 6a). When the fungal growth is examined under a microscope, the mycelium exhibits characteristics particular to *R. bataticola*, such as hyphae constriction, right angle branching (plate 6b). When the fungus grow older, spherical to irregular shaped sclerotia formed from mycelium (plate 6c) which further used to prepare the inoculation media for screening method.

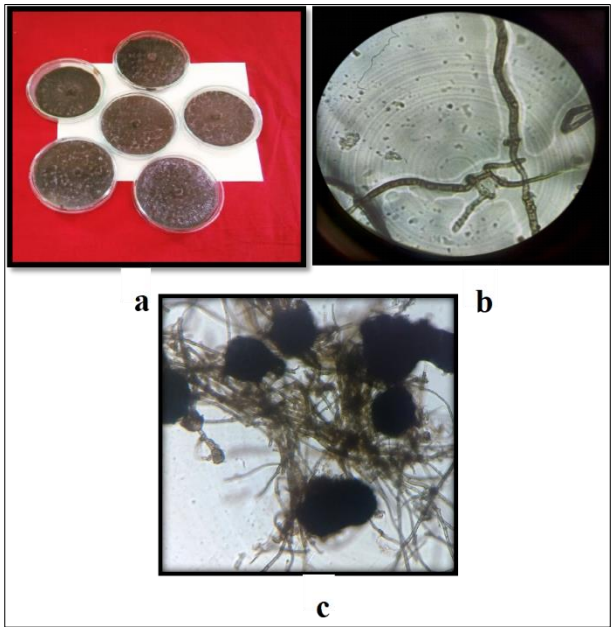

Plate 6: a- A pure culture of *R. bataticola* obtained from infected chickpea b- Microscopic view fungal hyphae c- Microscopic view of sclerotia

The study was conducted to standardize different screening techniques *viz.* seed inoculation, drenching of mycelium suspension, pot incolation and paper towel method were standardized for dry root rot disease exhibited significance difference (Fig. 1). JG-62 genotype of chickpea was used to standardize the screening technique. Maximum disease incidence (80%) observed in drenching of mycelium suspension method as compared to other methods *viz.* pot inoculation method (70 %) and paper towel method (76.70 %). Seed inoculation method showed minimum disease incidence (40 %) which exhibited low effectiveness of this method as compared to other method. Seed inoculation technique demonstrated significance difference with other methods. There were non significance difference observed among drenching of mycelium suspension, pot inoculation and paper towel method.

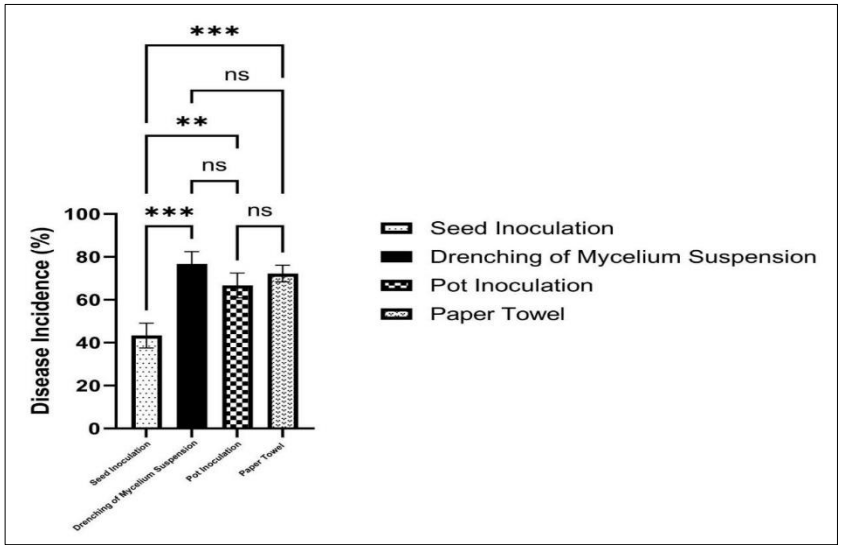

Fig 1: Evaluation of different screening method. Each values represent the mean ± SD of three replicates. *, **, ***represents the significance level at p<0.05, p <0.01, p<0.001, respectively. 'ns' – non significant

The morphological characters and colony character of mycelium and sclerotia demonstrated similarity with the result of [12, 14]. Some researcher evaluated the different screening techniques *viz.* paper towel method, sick field method and sick pot method at ICRISAT, Hyderabad [15,16]. Different inoculation techniques were evaluated for disease leaf blight of mungbean caused by *Macrophomina phaseolina* (Tassi) Goid [17]. Foliar inoculation technique was found significantly superior in causing leaf blight than seed and soil inoculation techniques. Soil inoculation method was reported as one of most effective method to develop symptoms of dry root rot and cause disease to screen chickpea genotypes [18]. The lowest incidence was recorded in pot inoculation technique (75.43 %). Soil infestation technique also evaluated to analyze symptoms of dry root rot and evaluate pathogenicity [19]. The effectiveness of different techniques *viz.* seed inoculation method, pot inoculation method or sick soil method, drenching of mycelium suspension, root dip technique and fine root cut mycelia suspension dip technique was studied in L-550 chickpea genotype. He observed highest incidence in fine root cut mycelia suspension dip technique (84.87 %) which was followed by root dip technique and drenching of mycelium suspension (83.78 and 80.96 %, respectively). Earlier it was reported that highest seedling mortality observed in soil inoculation method and minimum seedling mortality in root dip technique [8].

4. Conclusion

An experiment was carried out to evaluate the four artificial screening techniques for against dry root rot disease in chickpea caused by *Rhizoctonia bataticola*. Among all method, drenching of mycelium suspension method showed maximum disease incidence which was followed by paper towel, pot inoculation and seed inoculation method.

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