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Screening Chickpea Genotypes for Resistance to *Rhizoctonia bataticola* in Controlled Conditions

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ABSTRACT

Among the various biotic stresses, dry root rot caused by *Rhizoctonia bataticola* is becoming severe in most chickpea growing regions of India where the crop is grown under rain fed conditions causing 30-40 per cent yield loss. In this context, 520 chickpea genotypes were screened in the laboratory condition using Blotter Paper Technique to study the reaction of the genotypes to the *Rhizoctonia bataticola* and to identify resistance source for the disease. Among 520 genotypes, three were resistant *viz*., PG 06102, BG 2094 and IC 552137; 21 were moderate resistant *viz*., IC 15167, IC 2867, JAKI 9218, ICC 9023, ICC-14346, IC-269768, PG 01103, Pule 9801, KGD 120, NbeG 28, WR 315, IC-269488, IC 552198, IC 552178, IC 552132, IC 552320, IC 552214, IC 552232, CLH 29, IC 552102, IC 552224; 76 were moderate susceptible, 337 were susceptible and the rest eighty three were highly susceptible for dry root rot. The identified resistant genotypes may serve as potential donors in chickpea resistance breeding programme for dry root rot.

Key words: Blotter paper technique, Chickpea, Disease screening, Dry root rot resistance.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the oldest (earlier than 9500 BC) and widely cultivated pulse crops in over 50 countries of the world. Southwest Asia and the Mediterranean are the two primary centers of origin and Ethiopia, the secondary centre of diversity (Vavilov, 1926; 1951). Wild annual *Cicer* originated mainly in the Mediterranean regions having a wide eco-geographic range, differing in habitat, topographic and climatic conditions (Abbo *et al*., 2003; Berger *et al*., 2003). Globally, chickpea is grown in an area of 11.08 million hectares with the production of 9.77 millon tonnes and an average productivity of 881.9 kg ha^{-1}. It is the second most important pulse crop in the world in terms of area under cultivation after dry bean but ranks third in production following dry bean and peas. India is the largest producer of chickpea in the world sharing 65.25 and 65.49 per cent (FAOSTAT, 2010) of the total area (11.97 million ha) and production (10.89 million tonnes) with productivity of 920 kg/ha.

The realized productivity of this pulse crop is not appreciable due to various production constraints. The major being biotic stresses like diseases, insect pests and abiotic stresses like drought, extreme temperatures and salinity. Among the biotic stresses, chickpea is severely affected by *Fusarium* wilt, dry root rot, *Ascochyta* blight, collar rot, bacterial blight, filiform virus and root nematode causing economic yield loss (Nene and Sheila, 1996). Of late, dry root rot is becoming severe in most chickpea growing regions of India (Masood Ali and Shivakumar, 2001). Dry root rot of chickpea caused by *Rhizoctonia bataticola* is a soil borne fungus that survives in the soil in the form of sclerotia for long time. The fungus lacks fruiting bodies and spores. The mycelium is light brown, thick in which black sclerotia are formed.

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Although dry root rot disease is found in all chickpea growing areas, it is most severe in Central and South India, where the crop is grown under rain fed conditions causing 30-40 per cent yield loss. High day temperature (>30°C) and dry soil conditions at flowering and podding stage rapidly increase the severity of the disease (Gurha *et al*., 2003). The disease results in sudden drying of the plants in the field at flowering and poding, where as the leaves and stems of affected plants remain straw-colored. Roots dry, becomes brittle exhibiting shredding of bark and break easily. The disease appears suddenly when the ambient temperatures are between 25°C and 30°C (Haware, 1990).

Among the different approaches of prevention and management of diseases, the introgression of genetic resistance into cultivars is as an important and successful method as that is relatively inexpensive, biologically safe and sustainable for long term. The screening and evaluation of available germplasm is the first step in successful disease

resistance breeding programme to identify the sources of resistance which could be utilized further for development of resistant varieties. However, the research work carried out with respect to the identification of resistance sources to dry root rot of chickpea is meager. In this context, an effort was made to identify the resistant sources for dry root rot in chickpea.

MATERIALS AND METHODS

The present investigation started during *rabi* 2011, continued up to summer 2012-13 at the experimental plots of AICRP on Chickpea, Zonal Agriculture Research Station, Gandhi Krishi Vignana Kendra Campus, University of Agricultural Sciences, Bangalore.

Collection and isolation of the fungus

The chickpea plants showing typical symptoms of dry root rot (Isolate Rb1, Aghakhani and Dubey 2009) were collected from experimental plots of AICRP on Chickpea at GKVK, Bangalore (Fig 1A and 1B) and the samples were brought to the laboratory and washed thoroughly in running water. The plants were dried in shade for 3-4 days and preserved for further studies. Fungus was isolated following standard tissue isolation method under aseptic condition. Infected roots were cut into small bits of size 1-2 mm, surface sterilized in 1:1000 mercuric chloride solution for a minute and then washed repeatedly thrice in sterile distilled water to remove traces of mercuric chloride before transferring them to sterile potato dextrose agar [PDA] slants under aseptic conditions and incubated at 27 ±1°C.

Hyphal tip culture

Dilute mycelial suspensions (10^{-6} ml^{-1}) of the fungus were prepared in sterile distilled water. One ml of such (10^{-6} ml^{-1}) suspension was spread uniformly on two per cent water agar plates and observed for mycelial growth initiation under microscope. Such growth initiation was marked and mycelial bit transferred on PDA plates under aseptic conditions. The PDA plates were incubated at a temperature of 27±1°C and observed for fungal growth. No sectoring was observed in any of the plates after isolating them by hyphal tip culture technique. This method was followed for maintaining pure culture as the fungus is known to be highly heterozygous.



Fig 1A: Dry root rot infected plants collected from experimental plots of chickpea at GKVK.



Fig 1B: Photograph depicting symptoms of dry root rot infected plants.

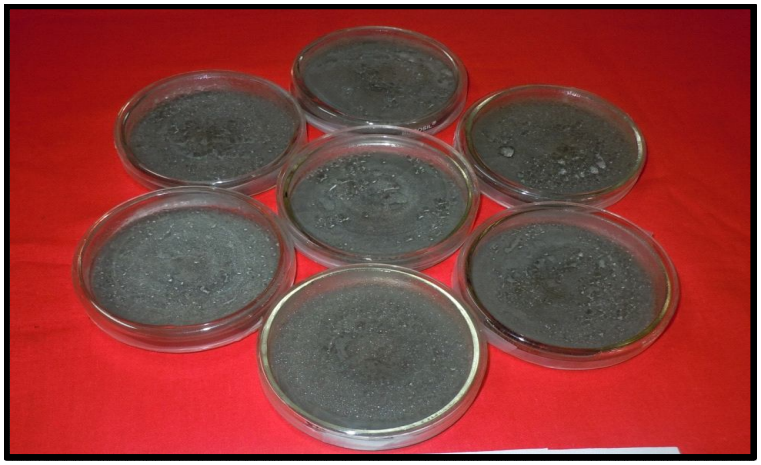


Fig 2: Sub culture of *Rhizoctonia bataticola* on Petri plates containing Potato dextrose agar.

Maintenance of culture

The fungus was sub cultured on Petri plates containing PDA (Fig 2) and allowed to grow at 27±1°C for ten days. Such plates were stored in refrigerator at 4°C and maintained by sub culturing at 20 to 25 days interval and used throughout the study.

Screening of germplasm lines for dry root rot resistance in laboratory condition

Five hundred and twenty chickpea germplasm lines procured from different national and international research institutes were screened in the laboratory condition using Blotter Paper Technique (Nene *et al*., 1981; Fig 3) to study the reaction of lines to *Rhizoctonia bataticola* infection and to identify the resistance sources for the disease. Potato Dextrose Broth (PDB) was used for culturing the fungus, *Rhizoctonia bataticola*. 250 ml of PDB was poured into 500 ml conical flasks and steam sterilized in autoclave at 15 PSI for 15 minutes. The flasks were then inoculated with the fungus and incubated at 35°C for seven days. The mycelial mat formed in the flask was removed and macerated in a waring blender along with distilled water for a minute. The inoculum was later collected in a beaker. In the mean time, chickpea seedlings were raised in polythene bags containing sterilized sand. One week old ten seedlings were uprooted and the roots were immersed in sterile water in order to remove the adhered soil particles. The seedlings were then immersed completely in the inoculum in a beaker for a minute. The seedlings particularly the root portion were then placed side by side on a blotter paper (45 cm x 25 cm with one fold) in such a way that only the cotyledons and roots are covered and the green tops of seedlings remained outside and then blotter paper was folded. One folder blotter paper contained the seedlings of one test line.

The folded blotter papers were then placed in trays and trays were placed in an incubator at 35°C for eight days. During the incubation period, twelve hour artificial light was provided and the blotters were moistened with sterile water every day. At the end of the incubation period, seedlings were examined for the extent of root damage and scored for the disease severity on 1- 9 rating scale (Nene *et al.,* 1981, Table 1).

Four resistant genotypes thus identified were again screened under green house condition in the pots containing the soil with *Rhizoctonia* fungus for reconfirmation of resistance along with the highly susceptible genotype L-550. Ten seeds per genotype were planted in each pot with two replications.

RESULTS AND DISCUSSION

Before initiating crop improvement programme in any crop, breeder should thoroughly evaluate, screen and understand the genetic architecture of the germplasm he is handling. Allard and Bradshaw (1964) indicated the need for thorough evaluation and utilization of germplasm for improvement of

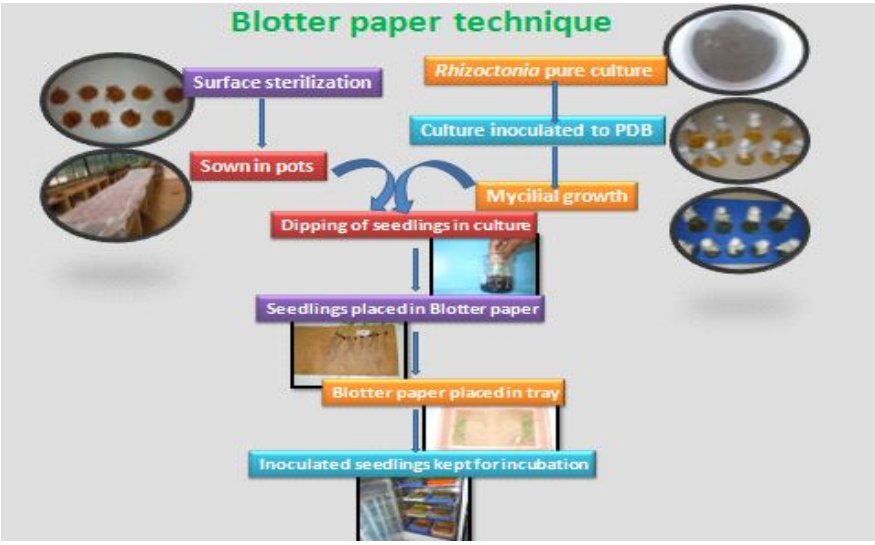


Fig 3: Flow chart of Blotter paper technique.

Table 1: Disease scoring scale for chickpea dry root rot disease.

Scale	Category	Symptoms
1	Resistant	No infection on roots
3	Moderately resistant	Very few small lesions on roots
5	Moderately susceptible	Lesions on roots clear but small, new roots free from infection
7	Susceptible	Lesions on roots many, new roots generally free from lesions
9	Highly susceptible	Roots infected and completely discolored

Table 2: Grouping of chickpea genotypes in response to dry root rot resistance under laboratory condition.

Disease scale	Response /Reaction	Number of Genotypes	Genotypes
1	Resistant	3	PG 06102, BG 2094, IC 552137.
3	Moderately resistant	21	ICC 2867, ICC 9023, ICC 14307, ICC 14346, ICC 15167, IC-269488, IC-269768, NBeG 28, KGD 120, PG 01103, JAKI 9218, IC-552337, IC 552214, IC 552132, IC 552102, IC 552198, IC 552224, IC 552232, IC 552320, IC 552178, Phule 9801.
5	Moderately susceptible	76	ICCV 08315, ICC 11550, ICC 14395, RSG 973, RSG 895, RSG 963, PBG 5, GNG 1958, GL 27104, GNG 1936, GNG 469, JG 23, BG 3031, Vijay, H 08-71, BG 3029, JGK 2003-304, GNG 1969, SC-RS-1, HK 94-134, IPCK 113, JGK 1, HK 07-234, Phule G 04305, IPCK 06-78, CSJK 42, GNG 2034, IPCK 06-56, GNG 1969, HK 06-152, Phule G 09316, GLK 28127, Kripa (Phule G 0517), JGK 13, CSJK 66, JGK 19, BG 3027, ICC 95, ICC 1052, ICC 1083, ICC 1356, ICC 2065, ICC 2072, ICC 2580, ICC 3761, ICC 4363, Phule 9801, IC-269254, IC-269306, IC-269489, IC-269542, IC-269761, CSJ 571, BGD 1051, BG 2050, ICCV 08121, ICCV 08123, IC-552082, IC-552274, IC-552170, IC-552117, IC 552131, IC 552057, IC 552196, IC 552311, IC 552241, IC 552125, IC 552261, IC 552189, IC 552165, IC 552209, IC 552302, IC 552255, RSG 811, SC RS-1, GL 26054.
7	Susceptible	337	ICCV 07107, ICCV 07306, ICCV 08113, ICCV 08117, ICCV 08120, ICCV 08323, ICCV 93706, ICCV 96854, ICC 1003, ICC 1376, ICC 2867, ICC 4969, ICC 9032, ICC 10803, ICC 10894, ICC 11088, ICC 11551, ICC 12249, ICC 12263, ICC 12267, ICC 12451, ICC 12969, ICC 14380, ICC 14431, ICC 14432, ICC 14441, ICC 14443, ICC 15167, ICC 15996, MGJG 11551, JG 9023, JG 251741, BG 212, RSG 991, CSJD 884, RSG 807, RSG 974, ICC 7323, NbeG 49, GJG 0809, Phule G 07102, BG 3013, GNG 1581, GL 27091, H 07-163, GL 26054 , GNG 1999, BDNG 2010-1, BGD 1068, JG 24, Phule G 0215-2, RVSSG 9, GJG 0907, CSJ 313, RVSSG 10, GNG 2065, Phule G 0204-4, GJG 0906, CSJ 0564, GNG 2064, IPC 07-19, H 08-13, BG 3004, IPC 08-11, H 08-18, GNG 1991, BG 3032, GJG 0922, BGD 1071, CSJ 697, H 08-25, ICC 8261, IP 06-127, IPCK 08-120, JGK 18, AKG 2002-1K, JG 28, GNG 2066, JG 27, IPC 07-56, PG 0101, RVSSG 8 IPC 2006-84 , JG 14, BGD 1072, RSG 888, CSJ 515, RSG 931, GNG 1996, BGM 572, BGD 1070, GJG 0910, BGM 572, BCP 60, CSJ 730, CSJ 513, JG 26, BG 3030, GJG 0904, BGD 1069, RSG 931, GNG 2002, GJG 0825, IPC 08-68, RSG 888, GNG 2085, IPC 07-09, BGD 1061 , CSJK 6, Phule G 0027, CSJK-1, GLK 26155, GNG 1888, RG 2003-24, GLK 26162, HK 08-231, CSJK 68, BG 3025, RVSSG 11, GNG 2112, HK 06-171, IPCK 07-62, RVSSG 12, Phule G 09311, CSJK 54, HK 08-212, GNG 2047, IPC 06-143, HK 06-163, GLK 26167, Phule G 09305, JGK 17, IPCK 08-136, BG 3012, HK 08-206, CSJK 70, CSJK 72, JGK 16, IPCK 08-130, GENESIS 90, FLIPPER, ICC 67, ICC 440, ICC 456, ICC 506, ICC 791, ICC 867, ICC 1098, ICC 1161, ICC 1180, ICC 1205, ICC 1230, ICC 1392, ICC 1397, ICC 1422, ICC 1431, ICC 1882, ICC 1915, ICC 1923, ICC 2210, ICC 2242, ICC 2263, ICC 2277, ICC 2482, ICC 2507, ICC 2593, ICC 2679, ICC 2720, ICC 2737, ICC 2919, ICC 2969, ICC 2990, ICC 3218, ICC 3239. ICC 3325, ICC 3362, ICC 3391, ICC 3410,ICC 3421, ICC 3512, ICC 3582, ICC 3631, ICC 3946, ICC 4093, ICC 4182, ICC 4418, ICC 4463, ICC 4495, ICC 4533, ICCV 2, ICCV 10, PG 03203, GAG 6731, AKG 04-18, IC-268980, IC-269309, IC-269388, IC-269411, IC-269426, IC-269440, IC-269485, IC-269522, IC-269540, IC-269541, IC-269643, IC-269756, IC-269763, GAG 0733, BGD 1047, GJ 0107, NbeG 1, RG 2003-23, CSJK 1, PG 9937, JSC 61,

Table 2: Continue....

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			GNG 1886, IPCK 441, HK 04-180, MNK-1, PKV Kabuli 4-1, PG 00110, PG 94092-1, PG 03110, HK 02-221, GBC 6, ICC-14346, ICC-16347, ICC-16348, KYABRA, ICC 14778, IC 15167, IC 2867, ICCX 0300-15-F4-P12-BP-BP, ICCX 0300-38-F4-P6-BP-BP, ICCX 0300-37-F4-P13-BP-BP, ICCX 0300-37-F4-P13-BP-BP, ICCV 07105, ICCV 07118, ICCV 08108, IC-269810, IC- 269757, ICCV 96030, ICC-13925, YORKER, ICCX 0200-46-F2-P8-BP-P1-BP-BP, IC-552124, IC-552291,IC-552287, IC 552277, IC-552186, IC-552182, IC-552298, IC-553471, IC-552171, IC-552220, IC-552155, IC-552339, IC-552109, IC-552066, IC-552222, IC 552322, IC 552136, IC 552150, IC 552187, IC 552203, IC 552297, IC 552218, IC 552058, IC 552193, IC 552342, IC 552130, IC 552284, IC 552124, IC 552327, IC 552157, IC 552325, IC 552316, IC, 552330, IC 552242, IC 552104, IC 552236, IC 552201, IC 552296, IC 552230, IC 552326, IC 552191, IC 552307, IC 552192, IC 552213, IC 552190, IC 552139, IC 552152, IC 552194, IC 552262, IC 552056, IC 552264, IC 552065, IC 552184, IC 552229, IC 552252, IC 552142, IC 552064, IC 552069, ICCC 37, ILC 1929, BGD 103, BG 256, HIR 70, BDNG 2003-1, A 1, CSJK 42, JG 62, BG 3012, HIR 55, ICC 4951, BGM 572, FLIP 03-35C, Ghaffa, K 850, FLIP 05-160C, FLIP 05-76C, FLIP 06-19C, CPS-1, JG 11, FLIP 06-157C, FLIP 06-155C, FLIP 05-73C, FLIP 06-158C, FLIP 02-23C, IPC-2004-98, FLIP-06-41C, FLIP-01-50C, FLIP 05-169C, FLIP 03-104C, JG-74, FLIP 03-99C, FLIP 06-114C, FLIP 05-83C, FLIP 05-24C.
9	Highly susceptible	83	BG 3024, PG 0105, GJG 0809, BG 3023, CSJ 303 , AKG 1001, GNG 1995, Phule G 0204-16, GNG 0921, NDG 1105, IPC 2006-77 , H 08-75, CSJ 753, GNG 2081, JG 25, GJG 0814, H 08-93, Phule G 09103, GNG 2068, JGK 2005-301, Vihar , GNG 2104, CSJK 74, Jimbour, ICC 283, ICC 637, ICC 708, ICC 762, ICC 1164, ICC 1194, ICC 1398, ICC 1510, ICC 1710, ICC 1715, ICC 2629, ICC 2884, ICC 3230, ICC 3776, ICC 3892, PG 95311, IC-269414, IC-269428, IC-269769, IC-269772, CSJ 563, PBC 101, CBS 101, JSC 62, ICC-16349, ICC-17256, GENESIS, GENESIS 508, ICCX 0300-37-F4-P18-BP-BP, ICCX 0300-37-F4-P27-BP-BP, WCG-2005-2, JKG 5303, IC-552251, IC-552226, IC-552112, IC 552312, IC 552254, IC 552153, IC 552301, IC 552323, IC 552205, IC 552239, IC 552063, IC 552060, IC 552154, BG 1105, GJG 0724, HIR 60, FLIP 03-26C, FLIP 06-40C, FLIP 06-156C, JGK-2003-304, FLIP 01-39C, L-550, FLIP 06-52C, FLIP 03-17C, FLIP 06-93C, FLIP 03-45C, FLIP 01-38C.



Fig 4: The extent of root damage due to *Rhizoctonia* infection clearly indicating the symptoms of dry root rot. A-Resistant, B-Moderate resistant, C-Moderate susceptible, D-Susceptible, E-Highly susceptible.

productivity in the field crops. Estimation of genetic variability parameters is the foremost step to be adopted in the source population if the breeding programme is aimed at improving economically important traits. The success of a crop improvement programme depends on the ability of the breeder to exploit genetic variability and select for yield associated and highly heritable components of phenotypic variation (Mather and Jinks, 1983).

Among many production constraints, biotic stresses play a major role, of which dry root rot of chickpea is emerging as severe disease in most of the chickpea growing regions of India where it is grown as rainfed crop (Masood Ali and Shivakumar, 2001). Though, different management practices available could be employed the importance of identifying and developing resistant varieties against diseases cannot be disputed in a crop like chickpea which is predominantly grown under residual moisture conditions and when the pathogen is soil borne. Host plant resistance is the most common and efficient method of disease control. Therefore, it is essential to identify resistant genotypes.

The extent of root damage due to *Rhizoctonia* infection and scoring for the disease severity on 1- 9 rating scale is represented in the Fig 4. Four genotypes out of 520 identified as a resistant in the laboratory screening, three genotypes *viz*., PG 06102, BG 2094 and IC 552137 showed resistant reaction to *Rhizoctonia* fungus in laboratory (Fig 5) as well as green house screening (Fig 6) indicating true genetic resistance of these genotypes. However, the genotype ICC 2867 showed moderate resistant reaction in the green house screening which earlier showed resistant reaction in the laboratory could have escaped the disease infection in the laboratory. Twenty one genotypes *viz*., ICC 2867, ICC 9023, ICC 14307, ICC 14346, ICC 15167, IC-269488, IC-269768, NBeG 28, KGD120, PG 01103, JAKI 9218, IC-552337, IC 552214, IC 552132, IC 552102, IC 552198, IC 552224, IC 552232, IC 552320, IC 552178 and Phule 9801 expressed very few



Fig 5: Genotypes PG 06102, BG 2094, IC 552137 and L 550 showed resistant and susceptible reaction to *Rhizoctonia bataticola* respectively.

small lesions on roots, hence they were grouped under moderately resistant category.

Further, seventy six genotypes exhibited small clear lesions on roots without infecting new roots and therefore grouped as moderately susceptible. Three hundred and thirty seven genotypes had many lesions on roots with new roots free from lesions and they were grouped as susceptible. The roots of remaining 83 entries were completely infected and discolored which clearly indicated the highly susceptibility of these genotypes to *Rhizoctonia.* A number

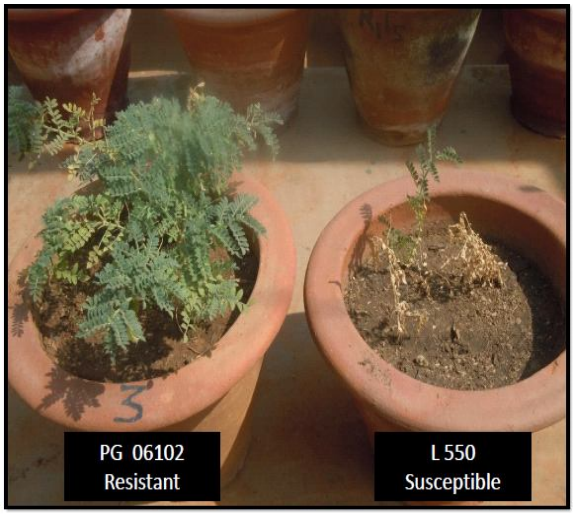


Fig 6: Confirmation of resistance for dry root rot in Green houe pots containing sick soil of *Rhizoctonia bataticola.*

of genotypes with moderate resistance reaction was reported by many workers (Pande *et al*. 2004, Muhammad Saifulla *et al*. (2011a and 2011b), Khan *et al*. 2013 and Nagamma *et al*. 2015). However, very few investigations have identified the resistant genotypes. Om Gupta and Anita (2006) observed the resistance reaction of H 99-264, PG 9425-5, PG 9425-9, HK 00297 and PG 97-313 genotypes for dry root rot out of 271 genotypes that were screened at in disease sick plot at JNKVV, Jabalpur, India. The genotypes *viz*., WCG 97-29, WCG 97-37, IPC 97-72 and H 00-256 (desi) showed less than 10% mortality for dry root rot. Jayalakshmi *et al*. (2008) reported two resistant genotypes from a two year screening of 12 chickpea genotypes in pot cultures. Further, several other workers *viz*., Mishra *et al*. (2005), Khan *et al*. (2012), Om Gupta *et al*. (2012), Khan *et al*. (2013), Nagamma *et al*. (2015) reported the resistance reaction of genotypes for dry root rot through blotter paper technique in the laboratory condition, pot culture method and sick plot screening. The resistant genotypes identified in the present investigation could be utilized as potential donors for future resistance breeding programme for dry root rot in chickpea.

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