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Purnima Singh
Department of Plant Pathology, College of Agriculture, Rajmata Vijyaraje Scindia Krishi Vishwa Vidyalaya, Gwalior, Madhya Pradesh, India

Reeti Singh
Department of Plant Pathology, College of Agriculture, Rajmata Vijyaraje Scindia Krishi Vishwa Vidyalaya, Gwalior, Madhya Pradesh, India

Corresponding Author:
Purnima Singh
Department of Plant Pathology, College of Agriculture, Rajmata Vijyaraje Scindia Krishi Vishwa Vidyalaya, Gwalior, Madhya Pradesh, India

Mycelial compatibility groups among the isolates of *Sclerotium rolfsii* associated with the collar rot disease of lentil

Purnima Singh and Reeti Singh

Abstract
Collar rot disease is emerging as one of the major impediments limiting the productivity of lentil. In the present study a total of nine isolates were obtained from diseased lentil crop from different areas of M.P. and mycelial interaction was studied among them to know the field population of pathogen. Out of 45 pairing only 20 combinations showed compatible reaction. Based on mycelial compatibility grouping, 44.44% vegetative compatibility was observed among the nine isolates.

Keywords: Mycelial compatibility groups (MCGs), lentil, collar rot, vegetative compatibility

Introduction
Lentil is a significant rabi pulse crop grown in India, which belongs to family Febaceae. In India it is grown in an area of about 1.49 million per ha with production and yield 1.61 million tons and 1006 kg per ha respectively (Anon, 2017-18) [1]. Moreover, it contributes share in Indian export basket as well, registering about 6.24% share in total pulse export during the year 2017-18. Diseases are one of the major impediments limiting the productivity of lentil. Among them collar rot caused by *Sclerotium rolfsii* is emerging as major disease of lentil due to changing climate.
Collar rot disease caused by soil borne fungus *Sclerotium rolfsii* Sacc. is an important disease, Sclerotium wide range of plants over 500 plant species (Punja and Grogan, 1983) [1]. The most characteristic symptom of collar rot disease is the appearance of white cottony mycelium growth on the collar region of the lentil plant just above the soil line, this often radiates over the soil surface, later on light brown to dark brown sclerotia were formed.
Based on mycelium interaction *S. rolfsii* isolates can be diverged into different MCGs (Mycelial compatibility groups). The MCGs helps in defining the field population of pathogen, facilitating genetic variation in pathogen (Kohn *et al*, 1991) [3]. The objective of the present study is to detect MCGs groups of the collected isolates from the different regions of M.P.

Material and Methods
Collection and isolation of Sclerotium isolates
The diseased lentil plants were collected from the nine different regions of the M.P. (Table no-1). Diseased collar part was cut into small pieces, sterilized for 1min in 1% sodium hypochlorite solution, and then washed with distilled water before culturing on PDA @ 25±2 ^{o}C.

Determination of MCGs
In order to determine MCGs of nine isolates obtained from different lentil growing regions of M.P. A 5mm disc was cut from a five day old actively growing culture from each isolate and placed in petridishes as pairings and cultured @ 25±2ºC on PDA medium (Punja and Grogan, 1983) [4]. The two isolates were kept 40mm apart from each other and incubated @ 25±2ºC on PDA medium for 10-14 days (Kohn *et al* 1991) [3].
The compatibility among each isolate was studied by presence or absence of barrage zone where hyphae collide. If the barrage zone was present, it was accepted as incompatible if not then compatible (Punja and Grogan, 1983) [4].

Table 1: Isolates of *S. rolfsii* collected from different lentil growing areas of M.P.

S. No.	Isolates	Year of collection	Host tissue	Location
1	Sr-1	2018	Collar region	Gwalior
2	Sr-2	2019	Collar region	Ashok nagar
3	Sr-3	2019	Collar region	Ganjbasoda
4	Sr-4	2019	Collar region	Morena
5	Sr-5	2019	Collar region	Bhind
6	Sr-6	2019	Collar region	Shivpuri
7	Sr-7	2019	Collar region	Jabalpur
8	Sr-8	2019	Collar region	Ratlam
9	Sr-9	2019	Collar region	Sehore

Results

There were 45 parings (Table no-2) of nine isolates, out of which only 20 combinations showed compatible reactions while remaining 25 combinations showed incompatible reactions. The compatible reactions shown by 20 amalgamations revealed that the two isolates paired were intermingled at the zone of interaction (fig: 1A). The coalescences which manifested incompatible reactions with each other have a thin band of dead mycelium formed in between the two pairings (fig: 1B). Based on this observation, 44.44% vegetative compatible groups were found among the isolates pairings. In all the incompatible reactions, Sclerotia were not formed. Sclerotia were formed at the border of the lytic zone of two isolates. In few combinations, on prolonged incubation it was observed that the interaction zone was broadened. The Sr-1 (Gwalior) isolate is only compatible with the Sr-9 (Sehore) isolate while as Sr-7 (Jabalpur) isolate showed incompatible reactions with all the other MCGs (Xie *et al*., 2014) [9].

Table 2: Mycelial compatibility interaction reaction shown by nine isolates associated with collar rot of lentil

Isolates	*Sr*-1	*Sr*-2	*Sr*-3	*Sr*-4	*Sr*-5	*Sr*-6	*Sr*-7	*Sr*-8	*Sr*-9
Sr-1	C	NC	NC	NC	NC	NC	NC	NC	C
Sr-2		C	C	C	C	C	NC	NC	NC
Sr-3			C	C	C	C	NC	C	C
Sr-4				C	C	C	NC	C	C
Sr-5					C	C	NC	C	C
Sr-6						C	NC	C	C
Sr-7							C	NC	NC
Sr-8								C	C
Sr-9									C

A **B**

Fig 1: Mycelial compatibility reaction A. Compatible reaction B. Incompatible reaction

Discussions

Our result is in accordance with the general conclusions drawn about *S. rolfsii* MCGs in earlier studies conducted by several workers; that the isolates of *S. rolfsii* can vary morphologically and genetically within the same MCG (Punja *et al*. 2001; Cilliers *et al*. 2000) [2, 6] and that unlikeness may be high within a field or across a region (Xie *et al*. 2014; Remesal *et al*. 2012) [7, 9].

Further high rate of non-compatible reactions in mycelia compatibility experiment reveals the extent of non-relatedness among the isolates. The death of mycelium at the barrage zones ascribes the involvement of toxins as well as the heterokaryotic condition of nuclei (Punja, 1985) [5]. There is need of further detailed study in this regard to unveil more details about the cause of mycelium deaths in incompatible reactions.

To the best of our knowledge there are no MCGs studies on the *S. rolfsii* of Lentil crop. However, few works has been performed on different crops of MCGs of *S. rolfsii* from same host and same region as well as different host and different regions and it has been reported that genetic variations have been present among different MCGs of different host crops (Unal *et al*, 2019) [8].

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