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Population Structure of *Sclerotinia sclerotiorum* (Lib.) de Bary Causing White Mold of Bean in Kashmir, India

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A B S T R A C T

The present investigation on population structure of *Sclerotinia sclerotiorum* (Lib.) de Bary casual organism of White mold disease of common bean (*Phaseolus vulgaris* L.) was carried by collecting diseased samples and sclerotia from different north Kashmir regions. A total of eighty pathogenic isolates of *Sclerotinia sclerotiorum* were maintained from four districts of North-Kashmir viz. Baramulla (36 isolates, B01-B36), Bandipora (08 isolates, N01-N08), Ganderbal (17 isolates, G01-G17) and Kupwara (19 isolates, K01-K19). These were subjected to mycelial compatibility testing and based on these tests a total of 22 Mycelial compatibility groups (MCGs) were formed. MCG-1 was the largest with 11 isolates followed by MCG-2 with 8 isolates and then by MCGs 3 and 4 each with 6 number of isolates. MCGs 5, 6, 7 and 8 consisted five isolates each while MCGs 9, 10, and 11 comprised three isolates each. MCGs 12, 13 were having three and 14, 15 two isolates each. MCGs 16, 17, 18, 19, 20, 21 and 22 were comprised of a single isolate each. Mostly, the isolates collected from adjacent localities were compatible. However, some MCGs also comprised distantly separated isolates. Therefore, the population of *S. sclerotiorum* is composed of many genotypes and while some MCGs are present in a particular area, many are distributed over larger areas.

Introduction

S. sclerotiorum (Lib.) de Bary is one of the most omnipresent, non-specific, ubiquitous necrotrophic pathogens which attacks a wide range of cultivated and wild plant species including canola (oilseed rape), mustard, alfalfa, soybean, field-bean, lentil, field pea, and sunflower. White mold or watery pod rot of bean, also known as Sclerotinia rot, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, limits the productivity of crop and reduces the market value of produce (Miklas *et al.*, 2006). It inflicts heavy losses to the bean crop which may reach 100 percent during pod formation (Steadman, 1983). The disease affects all plant parts *viz.* stem, leaves, pods etc. Infection usually commences at the junction of petioles and stem approximately 10-15 cm above the soil level. The brown water-soaked lesions appear first on the infected petioles and leaves and spread rapidly to the stem and branches.

Later superficial cottony mycelial growth of white mold is observed on infected petioles, stem and pods. As the infection advances, the pods show brownish discolouration and soft rot which results in dieback of branches. The fungus at this stage forms sclerotia which are initially white in colour but turn black at maturity (Ahanger *et al.,* 2006). In Kashmir the disease is increasingly posing threat to the profitable cultivation of bean especially in North Kashmir. Study of the population structure of the pathogen is the basic step to devise management strategies against the disease. A thorough attempt was made to study the population structure of the white mold pathogen from north Kashmir where the disease is increasing in its severity year after year.

Materials and Methods

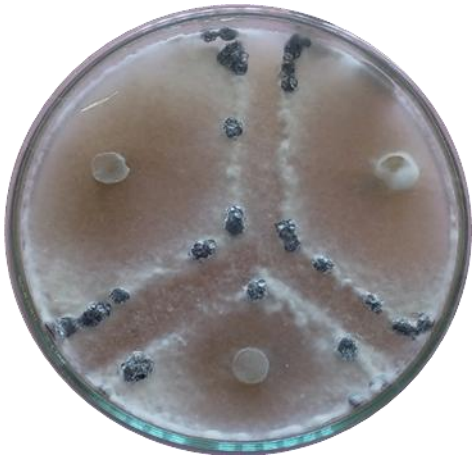
Naturally infected bean (*Phaseolus vulgaris* L.) plants bearing water-soaked lesions, cottony mycelial growth and/ or sclerotia on various plant parts were collected in polythene bags from the fields and brought immediately to the laboratory for investigation. Eighty pathogenic isolates were maintained on PDA in total from the collected diseased specimens from four North Kashmir districts using standard laboratory procedures and protocols. These included thirty six isolates from Baramulla (B01-B36), eight from Bandipora (N01-N08), seventeen from Ganderbal (G01-G17) and nineteen from Kupwara (K01-K19). The mycelial compatibility and incompatibility were determined as per method described by (Li *et al.,* 2009). Mycelial discs (5 mm diameter) taken from the edge of an actively growing colony of each isolate were placed triangularly on a PDA plate and incubated at 23 ± 2^{0}C. Mycelial reactions were recorded after 3-7 days of incubation. The reaction was considered incompatible, when an apparent line of demarcation or a barrage zone or a mycelia free zone was observed between the confronting paired isolates and as compatible, when no line of demarcation was observed.

Results and Discussion

Classification of isolates into MCGs is used routinely in many laboratories as a quick marker for genotyping *S. sclerotiorum* within populations. Table 1 shows the result of compatibility tests where 'C' represents the intersecting isolates are compatible while a blank cell represents incompatible reaction between the intersecting isolates. A coloumn always begins with 'C' as all the self-self pairings are compatible due to the fact that *Sclerotinia sclerotiorum* is a homothallic fungus. When two isolates are compatible with each other *i.e.* they form a confluent colony without any barrage zone between them, are assigned to a single MCG. However, when the isolates are incompatible with each other *i.e.* a barrage zone or a zone of clearance is formed the isolates are grouped under separate MCGs. Based on pairing of isolates as shown in table 1, eighty isolates of *S. sclerotiorum* were assigned into 22 Mycelial Compatibility Groups (MCGs) (Table 2). MCG-1 had the largest number of isolates 11, followed by MCG-2 with 8 isolates and then by MCGs 3 and 4 each with 6 number of isolates. MCGs 5, 6, 7 and 8 consisted five isolates each while MCGs 9, 10 and 11 comprised three isolateseach. MCGs 12, 13 were having three and 14, 15 two isolates each. MCGs 16, 17, 18, 19, 20, 21 and 22 were comprised of a single isolate each.

Grouping of 80 isolates into 22 MCGs on the basis of their compatibility test suggests a heterogeneous mix of genotypes of this pathogen in North Kashmir region. This agrees with previous reports on *S. sclerotiorum* MCG population structures on different crops (Kohn *et al.,* 1990; Hambleton *et al.,* 2002; Durman *et al.,* 2003; Kull *et al.,*

2004; Koga *et al.*, 2014). Koga *et al.*, (2014) while working on variability of *S. sclerotiorum* isolates from bean in Brazil reported 12 MCGs formed by 18 isolates.



a. Incompatible Reaction

b. Compatible Reaction

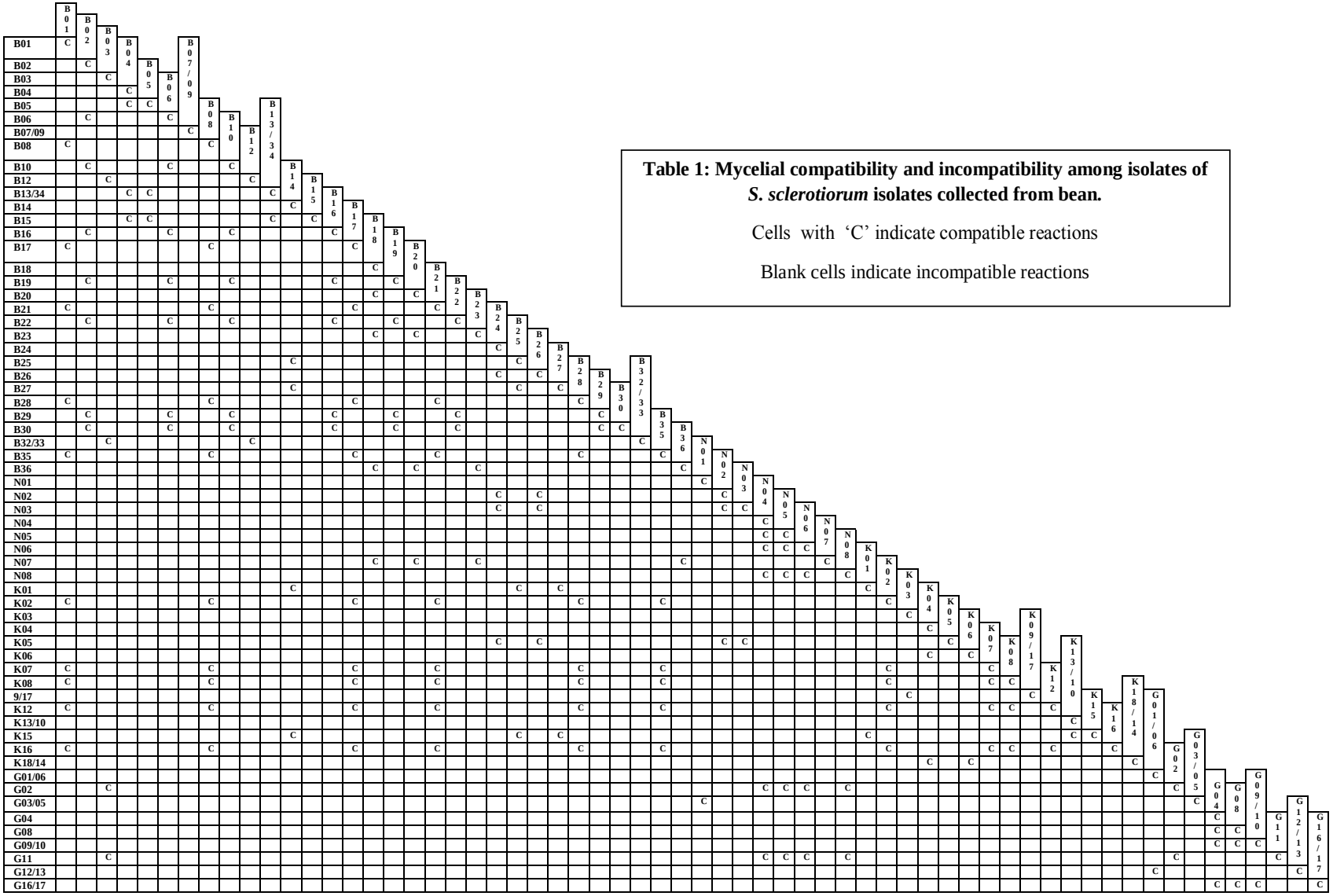
Table.2 Mycelial compatibility group constituent isolates and location

MCG	Isolate Codes	Location
01	B01, B08, B17, B21, B28, B35, K02, K07, K08, K12, K16	Baramulla and Kupwara
02	B02, B06, B10, B16, B19, B22, B29, B30	Baramulla
03	N04, N05, N06, N08, G02, G11	Bandipora and Ganderbal
04	G04, G08, G09, G10, G16, G17	Ganderbal
05	B04, B05, B13, B15, B34	Baramulla
06	B18, B20, B23, B36, N07	Baramulla and Bandipora
07	B14, B25, B27, K01, K15	Baramulla and Kupwara
08	B24, B26, K05, N02, N03	Baramulla, Kupwara and Bandipora
09	B03, B12, B32, B33	Baramulla
10	G01, G06, G12, G13	Ganderbal
11	K04, K06, K14, K18	Kupwara
12	K03, K09, K17	Kupwara
13	G03, G05, N01	Bandipora and Ganderbal
14	B07, B09	Baramulla
15	K10, K13	Kupwara
16-22	B11, B31, G14, G07, G15, K11 and K19	

Table 1: Mycelial compatibility and incompatibility among isolates of *S. sclerotiorum* isolates collected from bean.

Cells with 'C' indicate compatible reactions

Blank cells indicate incompatible reactions



The occurrence of single MCG from different North Kashmir districts, for example MCG-01 and MCG-7 throughout Baramulla and Kupwara regions, MCG-03 and MCG-13 from Bandipora and Ganderbal, MCG-06 from Baramulla and Bandipora and MCG-08 throughout Baramulla, Bandipora and Kupwara districts indicates the presence of same parental lineages of the pathogen across the region. This might be due to the trade of beans (seed and pulse, infested with sclerotia of this fungus), among the population of this region. Further this region is connected through blood relations among families and there is active exchange of beans among them leading to the dispersal of pathogen. Another reason may be the dispersal of ascospores through wind and water and sclerotia through irrigation water, these districts are having a good connectivity through rivers and canals. The results are supported by many findings for example, repeated recovery of MCGs in samples of *S. sclerotiorum* from canola made in Ontario in 1989 (Kohn *et al.*, 1991), western Canada in 1990, 1991, and 1992 (Kohli *et al.*, 1995; Kohli and Kohn, 1998), eastern Ontario and Quebec in 1999, and western Ontario in 2000 (Hambleton *et al.*, 2002). Similarly in the studies of (Kohli *et al.*, 1992) and Anderson and Kohn, (Anderson and Kohn, 1995) several clones were dispersed over large geographic areas, with clone 2 repeatedly isolated across 2000 km (recovered from Ontario, Manitoba, Saskatchewan, Alberta, and subsequently from cabbage in New York) over a 4-year period.

The heterogeneous mix of clones (MCGs) of the pathogen suggests that management strategies to be employed in a particular should be thoroughly developed taking into account the population structure and variability of the pathogen in that region. These MCGs can be used in morpho-cultural and pathogenic variability studies and germplasm screening programmes for any resistance source. It is also advised that while screening the bean germplasm for a resistant source against this pathogen more number of isolates should be employed.

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