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Rajeshwari Divya

Department of Plant Pathology,
College of Agriculture, IGKV
Raipur, Chhattisgarh, India

RK Dantre

Department of Plant Pathology,
College of Agriculture, IGKV
Raipur, Chhattisgarh, India

AS Kotasthane

Department of Plant Pathology,
College of Agriculture, IGKV.
Raipur, Chhattisgarh, India

Studies on Anastomosis Groups of Different Isolates of *Rhizoctonia Solani* Collected From Different Host

Rajeshwari Divya, RK Dantre and AS Kotasthane

Abstract

Anastomosis behaviour of different isolates with each other from different crops were investigated in different combinations and it was found that the type 2 reaction was observed in pairings between rice and maize with moongbean and groundnut isolates. Pairing of moongbean, soybean and groundnut isolates also resulted in similar type of reaction. When the soybean, rice and maize isolates were paired, they resulted in hyphal contact and apparent contact of wall but no membrane to membrane contact was observed and the mycelium grew in close contact with each other (reaction type 1). Pairings between moongbean, soybean and groundnut isolates resulted in type 2 reaction. It was found that all the isolates were self compatible and also showed self compatibility in the isolates from the same hosts.

Keywords: *Rhizoctonia solani*, Anastomosis, moongbean and isolates

Introduction

The genus concept of *Rhizoctonia* spp. was established by de Candolle (1815) (Sneh *et al.*, 1998). However, the lack of specific characters led to the classification of a mixture of unrelated fungi as *Rhizoctonia* spp. (Parmeter and Whitney, 1970; Moore, 1987). Ogoshi (1975) ^[17] enhanced the specificity of the genus concept for *Rhizoctonia* by elevating the characteristics of *R. solani* to the genus level.

The teleomorph of *Rhizoctonia* spp. belongs to the sub-division Basidiomycota, class Hymenomycetes. The anamorphs of *Rhizoctonia* are heterogeneous Moore (1987) placed the anamorphs of *Thanatephorus* spp. in *Moniliopsis*. She reserved the genus *Rhizoctonia* for anamorph of ustomycetous fungi which have septa with simple pores. *Moniliopsis* species have smooth, broad hyphae with brown walls, multinucleate cells, dolipore septa with perforate parenthesomes and teleomorphs in the genera *Thanatephorus* and *Waitea*. Affinity for hyphal fusion (anastomosis) (Parmeter *et al.*, 1969; Parmeter and Whitney, 1970; Ogoshi *et al.*, 1983a; Burpee *et al.*, 1980a) has been used to characterize isolates among *R. solani*, *R. zaeae*, *R. oryzae*, *R. repens* and binucleate *Rhizoctonia* spp. with *Ceratobasidium* teleomorphs. To date, isolates of *R. solani* have been assigned to 13 anastomosis groups (AG) and those of *R. zaeae* and *R. oryzae* have each been assigned to their own one group (Sneh *et al.*, 1998; Carling *et al.*, 1999, 2002c). Anastomosis reactions between hyphae of paired isolates of *R. solani* consist of several types; such as perfect fusion, imperfect fusion, contact fusion and no reaction (Matsumoto *et al.*, 1932). At present, four categories of anastomosis (C3 to C0) defined by Carling *et al.* (1996) have been accepted by many researchers.

These are useful for a better understanding of the genetic diversity of *R. solani* populations, because of the background genetically supported by vegetative or somatic compatibility (VC or SC) of confronted isolates (Mac Nish *et al.*, 1997). Anastomosis behaviour of the *R. solani* isolates was characterized. It was speculated that all the field isolates belonged to AG-1 group with its subgroups in leguminosae (AG-1 IB); soybean, moongbean and groundnut and the gramineae (AG-1 IA; paddy and maize). Based on variable reactions types three groups were speculated. Identity of "bridging isolated" to moongbean and groundnut isolates was given based on partial compatibility for reaction type 2 and can have an important role to play in gene flow within and between AGs in a particular field when mono cultured with paddy or alternated with other legume crops. (Vijayan and Nair 1985; Borthakur and Addy 1988; Basu and Gupta 1992; Singh *et al.* 1999; Sunder *et al.* 2003; Guleria *et al.* 2007) ^[12]. We studied the

Correspondence**Rajeshwari Divya**

Department of Plant Pathology,
College of Agriculture, IGKV
Raipur, Chhattisgarh, India

cultural and morphological variability within *R. solani* population in several rice growing areas in India.

Material and Methods

The present investigations were carried out in the Department of Plant Pathology, IGKV, Raipur. All *in vitro* studies on *Rhizoctonia solani* were conducted in the laboratory of Department of Plant Pathology, IGKV, and Raipur. Field trials were carried out at Research Farm, IGKV, Raipur.

In order to study anastomosis, the method described by Burpee *et al.* (1980) [4] was employed. Glass slides (3"×1") were cleaned with soap and were rinsed with distilled water. They were sterilized in hot air oven for 2-3 hours, by placing to slides in each well cleaned and air dried plastic plates sterilized in laminar air flow by giving UV rays. Slides were then coated with a thin layer of sterilized 2% water agar. Discs from actively growing cultures of *R. solani* were cut out with the help of cork borer from the edge and were placed 1 cm. apart on the coated slides and were then incubated for 36-48 hours (till the mycelium of the paired isolates came in close vicinity) at 27±2°C in BOD incubator. The area of hyphal contact was then stained with 0.5 percent lectophenol blue and a cover slip was placed to examined under the microscope at 10X and 40X magnifications. The branching habit of the isolates indicated the direction of growth and the hyphae could easily be traced back to their origin in order to ensure that anastomosis had occurred between mycelium of one isolate. All the 5 isolates were paired in possible combination. The grouping of isolates was done on the basis of reaction types.

Table 1: Following microscopic observation was as follows:

| Reaction Type | Descriptions |
|---------------|---|
| 0 | No anastomosis or repulsion. Neutral response. Occasional hyphal contact/ parallel growth but only for a short distance. |
| 1 | Hyphal contact and apparent contact of wall but no membrane to membrane contact. Both mycelium must grown in close contact with each other. |
| 2 | Wall to wall connection, pore formation and incompatible fusions of cytoplasm leading to death of the fusing and adjoining cells. |
| 3 | Fusion of wall and membrane. Compatible reaction. |

Result and Discussion

Anastomosis were tested by opposing isolates on 2% distilled water agar on microscopic glass slide tested in 9 cm petri dishes were selfed (*i.e.* isolates from a single host) and crossed (pairing of isolates from different hosts). All the isolates were self compatible and also showed self compatibility in the isolates from the same hosts. In the absence of a standard tester isolate "AG" identity to an isolate was not designated. However variable reactions types were observed between anastomosis behavior (Yokoyama and Ogoshi, 1984). Three groups were speculated based on reaction type between the isolates. Group 1- rice and maize with similar reaction (type 2) with moongbean and groundnut with similar anastomosis behavior when paired with isolates of rice, maize and soybean (reaction type 2) but showed partial compatibility (reactions type 2) with each other. Group 3 soybean showing reaction type 1 with rice and maize isolates but with moongbean and groundnut it was type 2 and thus was speculated as a different group. Selected combinations showing type 2 when paired in petri-plates showed inhibition zone while it was not seen in pairings of

reactions type 1. The occurrence of AGs in small areas or in a particular agricultural field is limited. Thus cultivated crops must exert a strong influence on the prevalence of a given AGs in certain areas or fields (Ogoshi and Ui, 1983). Ogoshi (1987) stated that the isolates of AGs -1 are mainly from the leguminosae (AGs 1 IB) and the gramineae (AGs 1 IA). Thus for the isolates collected in the present investigation, it was speculated that all the field isolated belonged to AGs 1 group. Three groups thus observed in the present investigation can be sub divisions based on the relative frequency of hyphal fusion and the reaction type, with fusion between two isolates from the same type in AG, being frequent, but that between two isolates from different types in AG being rare (Ogoshi, 1975). [17] To date, at least 13 AG have been described within the *R. solani* complex, with several sub groups. in each (Carling *et al.*, 1994) [6]. In the present investigation several type 2 reactions were observed. If type 2 reaction happened to be a partially compatible then there is a possibility that moongbean and groundnut isolates are the "bridging isolates" as these are partially compatible with rice, maize and soybean and the soybean happens to show group different from that of rice and maize. Yokoyama and Ogoshi(1984) also discussed the possibility of the existence of additional anastomosis groups and the possibility of "bridging isolates" *i.e.* those capable of anastomosing with number of two or more groups. A second possibility is if the reaction type 2 happens to be incompatible one than the four sub groups in AGs 1 are speculative as all the isolates when paired with each other, showed reaction type 2 except rice with maize which were the only isolates from two different host to show compatible fusion. Until further studies made to work out for the compatible of survivable of the anastomosing cells showing reactions type 2 and the existence of additional sub groups within a group with standard tester isolates is worked out, till then the interpretation of possibility of the "bridging isolates" and differences in anastomosis within a group remains speculative.

Anastomosis behaviour

Anastomosis behaviour of different isolates with each other from different crops were investigated in different combinations. All the isolates exhibited self compatibility *i.e.* fusion of wall and membrane and showed homologous anastomosis. Similarly when the isolates from the same hosts were paired compatibility was exhibited. Wall to wall connection, pore formation and incompatible fusion of cytoplasm leading to death of fusing and adjoining cells is a type 2 reactions which was observed in pairings between rice and maize with moongbean and groundnut isolates. Pairing of moongbean, soybean and groundnut isolates also resulted similar type of reaction. When the soybean, rice and maize isolates were paired, they resulted in hyphal contact and apparent contact of wall but no membrane to membrane contact was observed and the mycelium grew in close contact with each other (reaction type 1). Pairings between moongbean, soybean and groundnut isolates resulted in type 2 reaction. On the basis of anastomosis behavior of the isolates from different hosts three groups were speculated:

Group 1: Rice and maize Anastomosis behavior of rice and maize isolates were similar and resulted in reaction type 2 when paired with moongbean and groundnut while rice and maize showed compatible reaction (type 3).

Group 2: Moongbean and groundnut. Moongbean and groundnut isolates also showed similar anastomosis behavior with the isolates of rice, maize and soybean but showed

partial compatible reactions leading to the death of cells when paired against each other.

Group 3: Soybean. Anastomosis behavior of soybean isolates with rice and maize was of type 1 and with moongbean and groundnut it was type 2 and thus was speculated as a different group.

Few selected isolates were also paired in petri plates from different crops. Zone of inhibition was observed when moongbean and groundnut isolates were paired with the isolates of rice and maize. A similar type of interaction was observed in pairings made between moongbean, soybean and groundnut isolates. These observations are consistent to our microscopic observations on anastomosis behavior showing type 2 reaction. Zone of inhibition in a petri plate was not seen when soybean was paired with rice and maize isolates that gave type 1 reaction when observed for their anastomosis behavior. The reaction type of various isolates when paired with each other and against each other was given in table 9.

Table 2: Reaction type of various isolates.

| Isolates | Rice | Maize | Soybean | Moongbean | Groundnut |
|-----------|------|-------|---------|-----------|-----------|
| Rice | 2 | 2 | 1 | 2 | 1 |
| Maize | 2 | 2 | 1 | 2 | 1 |
| Soybean | 1 | 1 | 2 | 2 | 2 |
| Moongbean | 2 | 2 | 2 | 2 | 2 |
| Groundnut | 1 | 1 | 2 | 2 | 2 |

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