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ANASTOMOSIS GROUPS OF *RHIZOCTONIA SOLANI* KÜHN ISOLATES FROM POTATO IN PAKISTAN

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Abstract

Black scurf of potato, *Solanum tuberosum* L., caused by the fungus, *Rhizoctonia solani* is present in all the eight potato producing agro-ecological zones of Pakistan and *R.solani* anastomosis group (AG) 3 is the major cause of this disease in potato. The present investigations were conducted to study the occurrence of *R. solani* anastomosis groups (AGs) on this crop. During investigations, 127 *R. solani* isolates were recovered from potato tuber samples €collected during survey of disease from year 2001 to 2004. All of them were found multinucleate. Anastomosis group determination revealed 81.89% isolates belonging to AG 3 followed by AG 5, 8.66% and AG 4, 5.5%. Occurrence of AG 3 was the highest in all the potato production zones as compared to other AGs indicating *R. solani* AG 3 isolates pre-dominance on potato. The maximum number of AGs, 5 each, were found in zone 2, the Punjab province (AG1-1A, AG 3, AG 4, AG 5, and AG 9) and zone 7 comprising of parts of Northern areas of the country (AG 2-1, AG 2-2, AG 3, AG 4, and AG 5). It may be concluded from this study that several AGs are associated with black scurf disease. Anastomosis group 3 is the most common group of *R. solani* on potato here. Therefore, isolates of AG 3 may be used while breeding potatoes for host resistance to *R. solani*. This is the first report on anastomosis group determination of potato isolates of *R. solani* or any crop from Pakistan.

Introduction

Rhizoctonia canker or Black scurf caused by *Rhizoctonia solani* Kühn (perfect stage *Thanatephorus cucumeris* (A.B. Frank.) Donk) is a commonly occurring fungal disease of potato in Pakistan (Ahmad, 1998). The disease is a severe problem in all potato production agro-ecological zones of the country (Ahmad *et al.*, 1995 a-d and Khan *et al.*, 1995). *R. solani* was first time described by Kühn during 1858. It has been reported from all over the potato growing areas (Frank, 1986). This fungus is separated into sub-groups known as anastomosis groups (AGs). Coupling of isolates belonging to alike AG isolates results in hyphal fusion and they are regarded as genetically related or otherwise unrelated if showing somatic incompatibility. Isolates in which hyphal fusion occurs represents the same AG. Isolates of *R. solani* have been assigned to 12 AGs. AG 1, AG 2-1, AG 2-2, AG 3, AG 4, AG 5, AG 8 and AG 9 are the isolates recovered from potatoes in different parts of the world, among them AG 3 is the most commonly obtained (Abe & Tsuboki 1978; Chang & Logan, 1983; Carling & Leiner, 1986) and it is the major cause of black scurf and is relatively specific to potato. As host range and virulence of various AGs vary, information of these groups isolates in causation of a specific disease has become very helpful (Anderson, 1982; Bandy *et al*.,). Therefore, isolates of *R.solani* AG 3 needs to be used for inoculations in plant breeding for resistance to this fungus.

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Although, there is considerable information on AGs of *R. solani* attacking potato in other countries, significant gaps in our knowledge in Pakistan includes investigations on AGs of potato isolates of *R. solani* occurring in Pakistan. Hence, there was a need to understand the aspects of biology of black scurf of potato pathogen. Keeping in view the above background, extensive studies were planned with the objective to investigate the occurrence of anastomosis groups of *R. solani* on potato and nuclear condition in its isolates.

Materials and Methods

Determination of anastomosis groups: One hundred and twenty seven isolates of *R. solani* were recovered from 525 infected potato tuber samples collected during year 2001-04 from eight potato growing agro-ecological zones. All isolates were maintained on potato dextrose agar (PDA) medium at 10^{o}C and transferred from time to time to new medium. Anastomosis grouping was determined by the technique reported by Parmeter *et al*., (1969) and Balali *et al*., (1995). Each unidentified isolate of the fungus was subjected to hyphal anastomosis coupling by using AG 1-1A, AG 1-1B, AG 1-IC, AG 2-1, AG 2-2, AG 3, AG 4, AG 5, AG 6, AG 7, AG 8 and AG 9 tester strains of multinucleate *R. solani*. Anastomosis was tested by opposing isolates on PDA medium in 9 cm Petri dishes. Single unidentified isolate was tested against one known tester strain per dish. Four 5 mm diameter mycelial block from 4 days old culture of *R. solani* were plated 2-3 cm away from each other on PDA medium with a 5 mm diam., block of tester strain in the middle. The dishes were incubated at 25^{o}C (24-48 hours) by the time the growing hyphae of the isolate get in touch and overlapped with the hyphae of the tester strain. Each coupling was done at least two times.

A 0.5 to 1 cm^2 section detached from the area of contact was placed on a microscopic slide in a solution containing one drop of alkaline safranin 0 solution and one drop of 3.0% w/v potassium hydroxide (KOH). The safranin 0 solution comprised of 6 ml of 0.5% w/v safranin 0 in distilled water, 10 ml of 3.0% w/v KOH in distilled water and 5 ml of glycerin (Sneh *et al*., 1991). A cover slip was applied on it and observations were made under the microscope. Minimum five anastomosis reactions were taken into account for every sample.

For nuclear count a minute part of mycelium from 2 days old culture of each unknown *R. solani* isolate was placed in fixative (ethanol: glacial acetic acid, 3:1) in glass vials for 24 hours. Later, after giving washings in distilled water, the material was placed in hydrochloric acid (HCl) for one hour. Once more, the material was washed in distilled water in a Petri dish, and teased with a needle. A small segment of the mycelium was stained by adding a drop of safranin 0 solution and KOH (Sneh *et al.*, 1991) and the number of nuclei per cell were examined at X400 on Olympus, Japan, BH-2 series system microscope.

Results

Determination of nuclear condition of *R. solani* isolates and their anastomosis group: One hundred and twenty seven *R. solani* isolates were obtained from potato tuber samples collected during the disease survey. Zone-wise, isolates of *R. solani* obtained are presented in Table 1. Out of 127 isolates, 43 were obtained from samples of potato production zone 2, a major potato producing area comprising of parts of Punjab province, whereas, 10 isolates each were obtained from samples collected from zone 4 and 5 (parts of North West Frontier Province) and 8 (Balochistan province). Seventeen isolates were obtained from zone 1 (Sindh province), 11 from zone 3 (parts of Punjab and NWFP), 12 from zone 6 (parts of NWFP) and 14 from zone 7, Northern areas of Pakistan.

Table 1. *Rhizoctonia solani* isolates obtained from potato production agro-ecological zones of Pakistan.

Agro-ecological zone	Isolates obtained (number)
1	17
2	43
3	11
4	10
5	10
6	12
7	14
8	10

Table 2. Frequency of anastomosis groups of *R. solani* isolates

S. No.	Anastomosis group	Isolates (number)	Percentage
1.	AG 1-1A	1	0.79
2.	AG 1-1B	-	-
3.	AG 1-1C	-	-
4.	AG 2-1	2	1.57
5.	AG 2-2	1	0.79
6.	AG 3	104	81.89
7.	AG 4	7	5.51
8.	AG 5	11	8.66
9.	AG 6	-	-
10.	AG 7	-	-
11.	AG 8	-	-
12.	AG 9	1	0.79
	Total	**127**	

All the *R. solani* isolates tested for nuclear condition were found multinucleate. One hundred and four (81.89%) unknown isolates that anastomosed with the known tester strains of *R. solani* were members of AG 3 (Table 2). Of the remaining 23 isolates, 8.66% were members of AG 5, 5.51% AG 4, 1.57% AG 2-1 and 0.79% each belonging to AG 1-1A, AG 2-2 and AG 9. None of the isolate was from AG 1-1B, AG 1-1C, AG 6, AG 7 and AG 8.

Occurrence of AG 3 isolates was the highest in all the potato production zones as compared to other AGs (Table 3). AG 3 was found 100% in zone 4 followed by 91.67% in zone 6 and 90% in zone 8. Its minimum occurrence of 57.14% was found in zone 7. The maximum number of AGs, 5 each were found in zone 2 and zone 7. In zone 1, AG 3, AG 4 and AG 5 isolates were found (Table 3) and 5.88% isolates belonged each to AG 4 and AG 5. In zone 2, 81.40% AG 3 isolates were found, whereas, occurrence % of AG 1-1A and AG 9 isolates was 2.33% each. The occurrence of AG 4 and AG 5 isolates was 9.30% and 4.65%, respectively. In zone 3, AG 2-1, AG 3, and AG 5 isolates were found. The occurrence of AG 3 isolates was 81.82% followed by isolates of AG 2-1 and AG 5, 9.09% each. In zone 5, 70%, 10% and 20% isolates of AG 3, AG 4 and AG 5, respectively, were found, whereas, in zone 6, 91.67% isolates of AG 3 and 8.33% isolates of AG 5 were found. The occurrence of AG 4 isolates was the highest in zone 5 as compared to other zones.

Table 3. Occurrence of anastomosis groups of *R. solani* isolates in potato production agro-ecological zones of Pakistan.

S. No.	Anastomosis group	Occurrence (%)							
		Zones							
		1	2	3	4	5	6	7	8
1.	AG 1-1A	-	2.33	-	-	-	-	-	-
2.	AG 1-1B	-	-	-	-	-	-	-	-
3.	AG 1-1C	-	-	-	-	-	-	-	-
4.	AG 2-1	-	-	9.09	-	-	-	7.14	-
5.	AG 2-2	-	-	-	-	-	-	7.14	-
6.	AG 3	88.24	81.4	81.82	100	70	91.67	57.14	90
7.	AG 4	5.88	9.3	-	-	10	-	7.14	-
8.	AG 5	5.88	4.65	9.09	-	20	8.33	21.43	10
9.	AG 6	-	-	-	-	-	-	-	-
10.	AG 7	-	-	-	-	-	-	-	-
11.	AG 8	-	-	-	-	-	-	-	-
12.	AG 9	-	2.33	-	-	-	-	-	-

The occurrence of 21.43% AG 5 isolates in zone 7 was the highest, whereas, the occurrence of AG 3 isolates was the lowest as compared to other zones. Only isolates of AG 3 and AG 4 having occurrence of 90% and 10%, respectively, were found in zone 8.

Discussion

In the present survey, 127 multinucleate isolates of *R. solani* were obtained from potato tubers collected from 8 potato production zones. The isolates were identified for their nuclear condition and were found multinucleate. Anastomosis was determined by using 12 tester isolates of multinucleate *R. solani*. It is the first report on AG identification and AGs of *R. solani* occurring on potatoes from Pakistan.

Anastomosis group study revealed that 81.89% isolates belonged to AG 3 and they were equally common to all potato production zones. The most important non- AG 3 isolates found were members of AG 5 and AG 4 which comprised of 8.66% and 5.51%, respectively. The other AGs found belonged to AG 1-1A, AG 2-1, AG 2-2 and AG 9. The most common occurrence of *R. solani* AG 3 isolates on potato has also been reported from other countries of the world by many workers (Anderson, 1982; Bandy *et al*., 1984; Bolkan & Ribeiro, 1985; Ogoshi, 1985; Sherwood, 1969; Suresh & Mall, 1982). AG 1-1B, AG 1-1C and AG 8 have been found linked with potato in other geographic parts of the world were not isolated from tubers in our study. Bandy *et al*., (1988) concluded that it seems the production of sclerotia on tubers of non-AG 3 *R. solani* isolates occurs only incidentally, possibly due to local biotic and abiotic ecological factors. In the present studies, the same situation seems to be true.

Occurrence of AG 3 isolates, although, was common (57.14%) in agro-ecological zone 7 (NA) but it was the least as compared to other potato production zones. Carling & Leiner (1990) reported that *R. solani* is often described as a saprophytic survivor, but this explanation is perhaps less factual for *R. solani* AG 3 as compared to several other AGs and population of AG 3 turn down quickly in the absence of host but isolates that stay alive in the absence of potato in soil are able to maintain high levels of aggressiveness.

Thus, it seems that at locations like Northern Area where there is only summer season of potato and its cropping intensity is the least, the minimum occurrence of AG 3 apart from other factors seems due to this minimum cropping intensity of potato.

Conclusion

It is concluded from these investigations that several anastomosis groups are linked with black scurf disease of potato and they can infect potato crops in different time of year and locations. Anastomosis group 3 is the most common and an aggressive AG of *Rhizoctonia solani* on potato in Pakistan. Therefore, AG 3 isolates of *R. solani* may be used while breeding potatoes for resistance to black scurf.

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