PREVALENCE OF PATHOGENIC GROUPS OF Leptosphaeria maculans IN SERBIA

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Abstract

Among numerous pathogenic fungi on oilseed rape (Brassica napus L.), Leptosphaeria maculans belongs to the most important disease agent in many countries (Canada, Australia, Germany, France, Great Britain), where this plant species is extensively grown. In Serbia, very little is known about this pathogenic species and even less about the current distribution of pathogenic groups (FGS) of L. maculans. Based on literature and experimental studies isolates are classified as to FGC (FGC known as L. maculans). A total of 119 isolates were tested originating from Serbia on differential varieties of oilseed rape (Westar, Quinta and Glacier). All 4 FGC have been observed in the population collected during 2009 and 2010. Key words: oilseed rape, Leptosphaeria maculans, L. maculans biglobosa, pathogenicity, groups prevalence.

Introduction

Leptosphaeria maculans (D.Cke) Crous & de Hoog (syn. Phoma lingam (L.) Sacc.) is a soil-borne, late maturing pathogenic fungus. This fungus is an important disease agent of oilseed rape (Brassica napus L.) in many countries (Canada, Australia, Germany, France, Great Britain), where the plant species is extensively grown. In Serbia, very little is known about this pathogenic species and even less about the current distribution of pathogenic groups (FGS) of L. maculans. Based on literature and experimental studies isolates are classified as FGC (FGC known as L. maculans). A total of 119 isolates were tested originating from Serbia on differential varieties of oilseed rape (Westar, Quinta and Glacier). All 4 FGC have been observed in the population collected during 2009 and 2010. Key words: oilseed rape, Leptosphaeria maculans, L. maculans biglobosa, pathogenicity, groups prevalence.

Materials and Methods

Experimental plants and in vivo/on vivo culture

Infected plants of oilseed rape were collected during 2008 and 2010. In the region Vojvodina, Serbia, the infected plants (late stage), upper them, leaf, crown, (flowering, blooms) with well-developed clinical disease, were used for isolation of fungi. Fragments of diseased tissues were immersed in 10% solution of sodium hypochlorite for 5 min. and then washed with sterile water and dried naturally under controlled conditions. After drying, the fragments of diseased tissue were placed in a culture medium of potato dextrose agar (PDA) (Difeo, DIFCO, U.S.A.) which had previously been rinsed in PDA solution. The plates were incubated in a humid chamber at 20°C. After 5–6 days, the mycosphere was taken off using a sterile scalpel and transferred to a liquid nutrient culture (Golenko, Belgrade, Serbia). Field dishes with such substrate were placed in a thermostat at a temperature of 22°C ± 1°C. After 5–6 days, the mycosphere was grasped gently by a sterile scalpel and microscopically pure cultures were obtained as follows: fungi that were released from the mycosphere, originating from the culture media, were transferred using a sterile needle tip into plastic tubes, which had previously been rinsed in PDA solution, transferred to the liquid nutrient culture, and kept under aseptic conditions for 3 days. After 3 days, the surface of the liquid culture was cut with a sterile needle tip, and transferred the mycosphere to a new liquid nutrient culture, and kept under aseptic conditions for another 3 days. After 3 days, the mycosphere was transferred to a medium of potato dextrose agar (PDA) (Difeo, DIFCO, U.S.A.) which had previously been rinsed in PDA solution and placed in a humid chamber at 20°C. After 5–6 days, the mycosphere was cut off using a sterile scalpel and transferred to a liquid nutrient culture (Golenko, Belgrade, Serbia). Field dishes with such substrate were placed in a thermostat at a temperature of 22°C ± 1°C. After 5–6 days, the mycosphere was grasped gently by a sterile scalpel and microscopically pure cultures were obtained as follows: fungi that were released from the mycosphere, originating from the culture media, were transferred using a sterile needle tip into plastic tubes, which had previously been rinsed in PDA solution, transferred to the liquid nutrient culture, and kept under aseptic conditions for 3 days. After 3 days, the surface of the liquid culture was cut with a sterile needle tip, and transferred the mycosphere to a new liquid nutrient culture, and kept under aseptic conditions for another 3 days. After 3 days, the mycosphere was transferred to a medium of potato dextrose agar (PDA) (Difeo, DIFCO, U.S.A.) which had previously been rinsed in PDA solution and placed in a humid chamber at 20°C. After 5–6 days, the mycosphere was cut off using a sterile scalpel and transferred to a liquid nutrient culture (Golenko, Belgrade, Serbia). Field dishes with such substrate were placed in a thermostat at a temperature of 22°C ± 1°C. After 5–6 days, the mycosphere was grasped gently by a sterile scalpel and microscopically pure cultures were obtained as follows: fungi that were released from the mycosphere, originating from the culture media, were transferred using a sterile needle tip into plastic tubes, which had previously been rinsed in PDA solution, transferred to the liquid nutrient culture, and kept under aseptic conditions for 3 days. After 3 days, the surface of the liquid culture was cut with a sterile needle tip, and transferred the mycosphere to a new liquid nutrient culture, and kept under aseptic conditions for another 3 days. After 3 days, the mycosphere was transferred to a medium of potato dextrose agar (PDA) (Difeo, DIFCO, U.S.A.) which had previously been rinsed in PDA solution and placed in a humid chamber at 20°C. After 5–6 days, the mycosphere was cut off using a sterile scalpel and transferred to a liquid nutrient culture (Golenko, Belgrade, Serbia).

The distribution of pathogenic groups (FGC, FGC, FGC, FGC) in Serbia has been examined by the method of each (1991), Mengato et al. (1991) and Chen and Chen (2003). Seeds were dissected by dipping in 3% solution of sodium hypochlorite (NaOCl) for 3 to 5 min. and then washed with tap water and dried at room temperature under controlled conditions. The success of dissection was tested on PDA nutrient medium. For seeds dissected, 5–6 replicates were used. The seeds were not dissected and not dissected seeds of undamaged, in each box 10 seeds were placed 5 seeds, then prepared leaf disks were placed in a thermostat at 25°C ± 1°C. After 15 days, the leaves were covered with visual, microscopic examination of the seeds and subsitutions were made for plates in a generation chamber (Golenko, Belgrade, Serbia). Infected seeds were heated in plastic containers with a diameter of cells 4 cm, which were filled with 30% H2O2 and baked and heated at 70°C for 15 min. After 3 days, the heated seeds were covered with sterilized paper and placed in a humid chamber at 20°C. After 5–6 days, the leaves were covered with visual, microscopic examination of the seeds and subsitutions were made for plates in a generation chamber (Golenko, Belgrade, Serbia). Infected seeds were heated in plastic containers with a diameter of cells 4 cm, which were filled with 30% H2O2 and baked and heated at 70°C for 15 min. After 3 days, the heated seeds were covered with sterilized paper and placed in a humid chamber at 20°C. After 5–6 days, the leaves were covered with visual, microscopic examination of the seeds and subsitutions were made for plates in a generation chamber (Golenko, Belgrade, Serbia). Infected seeds were heated in plastic containers with a diameter of cells 4 cm, which were filled with 30% H2O2 and baked and heated at 70°C for 15 min. After 3 days, the heated seeds were covered with sterilized paper and placed in a humid chamber at 20°C. After 5–6 days, the leaves were covered with visual, microscopic examination of the seeds and subsitutions were made for plates in a generation chamber (Golenko, Belgrade, Serbia). Infected seeds were heated in plastic containers with a diameter of cells 4 cm, which were filled with 30% H2O2 and baked and heated at 70°C for 15 min. After 3 days, the heated seeds were covered with sterilized paper and placed in a humid chamber at 20°C. After 5–6 days, the leaves were covered with visual, microscopic examination of the seeds and subsitutions were made for plates in a generation chamber (Golenko, Belgrade, Serbia). Infected seeds were heated in plastic containers with a diameter of cells 4 cm, which were filled with 30% H2O2 and baked and heated at 70°C for 15 min. After 3 days, the heated seeds were covered with sterilized paper and placed in a humid chamber at 20°C. After 5–6 days, the leaves were covered with visual, microscopic examination of the seeds and subsitutions were made for plates in a generation chamber (Golenko, Belgrade, Serbia).

Results and Discussion

From a total of 119 isolates isolated in 2009 and 2010 in Vojvodina, Serbia, on the basis of pathogenic groups (FGC), 46 isolates were tested on the differential varieties of oilseed rape (Westar, Quinta and Glacier). All 4 FGC have been observed in the population collected during 2009 and 2010. Key words: oilseed rape, Leptosphaeria maculans, L. maculans biglobosa, pathogenicity, groups prevalence.

This study showed that all pathogenic groups of L. maculans are present in the oilseed-producing regions of Serbia (Vojvodina). Similar results of presence of FGC (pathogenic groups) in other countries were stated by Mengato et al. (1991), Chen and Chen (2003). Pathogenic groups within the population of group A are not equally represented in all growing regions of oil seed rape. In Canada (Canada) and Mexico (Mexico) FGC was represented by 80%, FGC by 15%, and according to the estimation, FGC belongs to a new group marked with FGC. The result of Radovic et al. (1997), isolates from Western Canada, showed that FGC belonged to a new group marked with FGC. The result of the present research was consistent with the statements of Chen and Chen (2003) that new FGC is discovered, for now in the U.S. and Canada in Sweden FGC and FGC were dominant, while FGC was not observed and FG was not observed in very low percentage (Chen and Chen, 2003), the same authors stated that there is no correlation between pathogenic groups and sites of isolation and that FGC is more observed at the top of the tree, while in Serbia FGC is isolated from the crown and FG (Kuska et al., 2003).

In Australia FGC and FGC are dominant, but FGC is present in very low percentage while FG is not detected (Kulk et al., 1991), based on the analysis of the presence of FGC and FGC in the country is very similar to the result of Kulk et al. (1997). In Canada, considering that all four groups were detected in Serbia (Also confirmed by PCR analysis—data not shown). These results show that FGC presence can provide reliable information about the dynamics and pathogenicity of L. maculans in the producing region.