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Temperature sensitivity and freezing resistance among isolates of *Typhula ishikariensis* from Russia

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SUMMARY

Frost tolerance of mycelia and sclerotia were determined in Russian varieties from Moscow and Novosibirsk (South Siberia) of *Typhula ishikariensis*. When mycelia and sclerotia of isolates from Moscow and Novosibirsk were rapidly frozen to –40°C in a program freezer, mycelia of isolates of Moscow were destroyed and the regrowth from sclerotia was delayed, whereas the mycelial regrowth of isolates from Novosibirsk were not affected and sclerotia of theses isolates survived the freezing stress. These results suggested that isolates of Novosibirsk are more resistant to rapid freezing stress than those from Moscow. Isolates from Novosibirsk are well adapted to climatic condition in South Siberia.

Key words: freezing resistance, Russia, Siberia, *Typhula ishikariensis*.

YFIRLIT

Hitanæmni og frostþol rússneskra stofna af Typhula ishikariensis

Frostþol myglu og hvílugróa var rannsakað í rússneskum stofnum af *Typhula ishikariensis* frá Moskvu og Novosibirsk (Suður-Síbería). Þegar mygla og hvílugró af einangrunum frá Moskvu og Novosibirsk voru fryst hratt niður í –40°C í hitastýrðum frysti eyðilagðist mygla af einangrunum frá Moskvu og endurvexti frá hvílugróum seinkaði, en þetta hafði engin áhrif á vöxt myglu frá Novosibirsk og hvílugró frá þessum einangrunum lifði af frostálagið. Niðurstöðurnar benda til að einangranir frá Novosibirsk séu þolnari gagnvart hraðfrystingu en einangranir frá Moskvu. Einangranir frá Novosibirsk eru vel aðlagaðar veðurfari í Suður-Síberíu.

INTRODUCTION

Snow mold fungi are psychrophilic or psychrotrophic fungal pathogens that infect winter cereals grown in northern hemisphere. *Typhula ishikariensis* S. Imai has evolved high adaptability to different winter climates among the snow molds (Matsumoto, 1992, 1995). *T. ishikariensis* from European part of Russia, Baltic countries and Northern Ukraine have the same genetic background. They mate with the Japanese Biotype A and Norwegian group I (Tkachenko *et al.*, 1997). Recently, *T. ishikariensis* were collected in the Novosibirsk district, Southern Siberia (Tkachenko, unpublished results).

Novosibirsk district has snowcover for 165 days and the average snow depth is 30–35 cm. Minimum air temperature in winter season reaches –40 to –45°C. The freezing of soil begins at the end of October to early November, before snow fall. In cold years, the depth of soil frost can reach 3 m (Ponamorenko, 1998).

Freezing resistance is an important factor that correlates with the geographical distribution of *T. ishikariensis* in Norway (Hoshino *et al.*, 1998). Isolates from Novosibirsk are assumed to have better adaptation to low temperatures than isolates from European parts of Russia. In this study we aim to elucidate possible differences in low temperature adaptation between isolates from European parts of Russia (Moscow) and isolates from Novosibirsk district (South Siberia).

MATERIAL AND METHODS

Fungal strains and media

Two strains of each group of isolates (Moscow: 92Tr-13, 93Tul-1 and Novosibirsk: 98N3, 98N5) were used in the experiments. Isolates 92Tr-13 and 93Tul-1 were collected from *Tulipa gesneriana* in Moscow in 1992 and 1993, respectively (Tkachenko *et al.*, 1997). Isolates of 98N3 and 98N5 were collected in Novosibirsk in 1998 from *Stellaria media* and *Phleum pratense*, respectively. Cultures were maintained on potato dextrose agar (PDA) slants at 4°C.

Mating experiments

Mating experiments followed the methods of Bruehl *et al.* (1975) with slight modification (Matsumoto, 1989). We used 8 monokaryons in mating experiments (Table 1).

Growth temperature

Mycelial discs, 5 mm in diameter, were cut from the margin of an actively growing colony, transferred to the centers of PDA plates (9 cm in diameter), and incubated at 4 different temperatures from 0 to 15°C, in duplicate. After 1, 2 and 3 weeks of incubation, the colony diameters were determined. The linear mycelial growth rate per day was calculated after the initial lag period.

Determinations of the mycelial and sclerotial freezing resistance

Mycelial and sclerotia freezing resistance were determined by the regrowth of mycelia after freezing stress (Hoshino *et al.*, 1998). A mycelial disc of 5 mm in diameter (cut from the margin of an active growing colony) and wet sclerotia, were surface-sterilized by 70% EtOH, and placed on a PDA plate of 2.5 cm in diameter and frozen to –40°C in a program freezer at a fast cooling rate of 20°C/h. After freezing, the mycelial discs and sclerotia were thawed at 2°C for 16 h, transferred to fresh PDA plates, and incubated at 2 and 10°C. Mycelial growth was observed daily up to 30 days.

Table 1. Mating reaction of Siberian *Typhula ishikariensis*.
1. tafla. Pörunarviðbrögð Typhula ishikariensis *frá Síberíu.*

Tester monokaryons		Tested isolates 98N3	98N5
92-32m1	(European part of Russia)	+	+
2-5BS-1	(Norway, group I)	+	+
4-3S-5	(Norway, group II)	–	–
6-1BS-4	(Norway, group III)	+	+
PR7-6-7	(Japan, biotype A)	+	+
PR9-4-3	(Japan, biotype A)	L	–
35-8	(Japan, biotype B)	–	–
8-2	(Japan, biotype B)	–	–

+: Vigorous hyphae with clamps produced.
–: Vigorous hyphae without clamps.
L: Lateral dikaryotization.

RESULTS AND DISCUSSION

Mating compatibility

Isolates from Novosibirsk mated with the biological species I, as the isolates from the European part of Russia, the Norwegian groups I, III and the Japanese biotype A (Table 1). Previous results showed that all isolates from European part of Russia, Baltic countries and Northern Ukraine mated with Norwegian group I and Japanese biotype A. However, isolates from European part of Russia, Baltic countries and Northern Ukraine were incompatible with Norwegian group III (Tkachenko *et al.*, 1997). These results suggest that Novosibirsk isolates has some common genetic background with strains belonging to Norwegian group III.

Growth temperature

Two isolates from Moscow and one isolate from Novosibirsk isolate (98N5) had the same optimum growth temperature, 10°C (Figure 1). Another isolate from Novosibirsk (98N3) showed maximum growth rate at 5°C. Isolates from Novosibirsk had a slower growth rate than the isolates from Moscow at optimum temperature. Both groups of isolates showed almost same optimum temperature as previously reported from Russian isolates (Potatosova, 1960).

The Norwegian group III strains had an optimum temperature at 4°C. The mycelial growth of group III was arrested at relatively moderate temperature (above 10°C) and formed a feather-like colony (Matsumoto *et al.*, 1996; Hoshino *et al.*, 1997). Isolates from Novosibirsk had some common genetic background with Norwegian group III strains. However, isolates from Novosibirsk did not show these changes of mycelial morphology at 10 and 15°C. They kept their ability to grow in a relatively moderate temperature range (above 10°C). Thus it is thought that isolates from Novosibirsk, which have acquired the ability of growth in a relatively moderate temperature range, are adapted to the spring of continental climate in south Siberia.

Freezing resistance

Figure 2 shows the regrowth of all four isolates at 10°C 21 days after the rapid freezing. Isolates from Novosibirsk resumed growth after freezing. Isolates from Moscow, however, did not regrow at this temperature. These results suggest that resistance to freezing stress is an

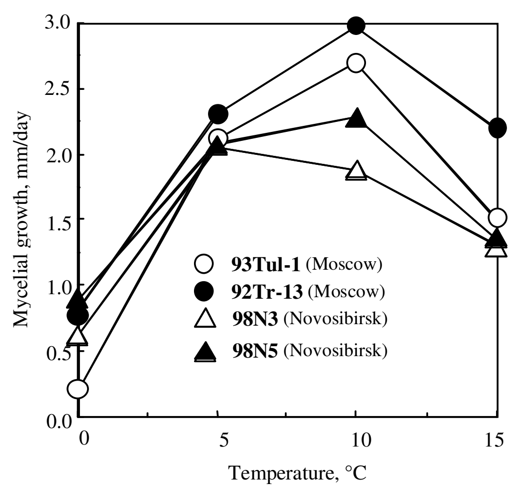

Figure 1. Effect of temperature on mycelial growth of Russian *Typhula ishikariensis*.
1. mynd. Áhrif hitastigs á mygluvöxt Typhula ishikariensis *frá Rússlandi*.

important factor for the survival of Novosibirsk isolates in south Siberia, since fungi under snow cover are subject to a temperature of around 0°C and rapid temperature changes in the Novosibirsk district. Similar results were obtained from the freezing resistance tests of sclerotia (Figure 3). Thus, it is thought that Siberian isolates, which have acquired the ability of frost resistance, are adapted to the severe winter environment in Siberia. These results suggest that isolates from Novosibirsk and the Norwegian group III isolates have a similar survival strategy against severe winter climate.

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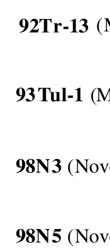

Figure 2. Effect of freezing stress on mycelial growth of Russian *Typhula ishikariensis*. Mycelial growth at 10°C, 21 days after freezing stress.
2. mynd. Áhrif frostálags á mygluvöxt Typhula ishikariensis *frá Rússlandi. Mygluvöxtur við 10°C og 21 dag eftir frystingu.*

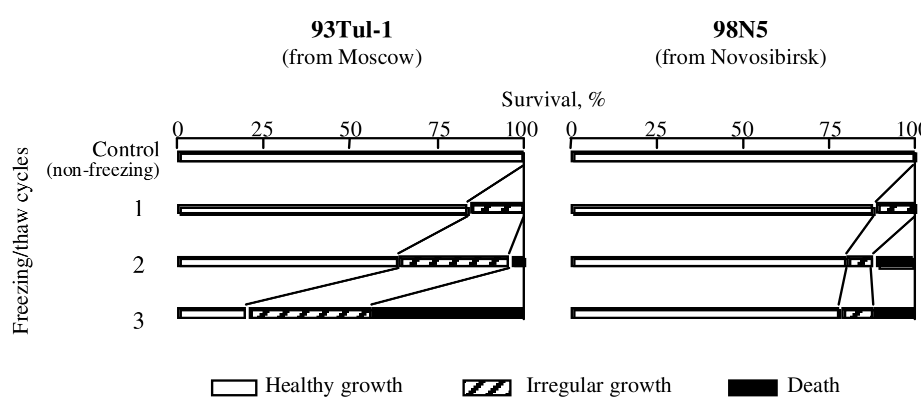

Figure 3. Freezing resistance of sclerotia of Russian *Typhula ishikariensis*, exposed to freeze/thaw cycles.
3. mynd. Frostþol eftir frystilotur hvílugróa af Typhula ishikariensis *frá Rússlandi.*

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