Effect of common bunt on the frost resistance and winter hardiness of wheat (*Triticum aestivum* L.) lines containing Bt genes

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Summary

In order to determine the effects of bunt inoculation on frost resistance and winter hardiness in lines containing resistance genes, the bunt [*Tilletia foetida* (Wallroth) Liro, *T. caries* (DC.) Tulasne] susceptibility of wheat lines containing bunt resistance genes Bt1 to Bt10 and the effect of the year on the degree of infection were studied over six years from 1991 to 1997 in an artificial inoculation nursery. Uninoculated and artificially inoculated wheat plants were tested for frost resistance in the phytotron in 1995 and in the field in boxes in three years from 1994/95 to 1996/97. The line with Bt10 was very resistant, lines with Bt5, Bt6, Bt8 and Bt9 were resistant, the line with Bt4 was moderately resistant, those with Bt2 and Bt3 were moderately susceptible, the line with Bt1 was susceptible and the line with Bt7 was very susceptible to the local bunt population in Hungary. Bunt incidence also varied over years. The frost resistance of the Bt lines was generally lower after bunt inoculation than that of uninoculated plants. The increased frost kill in inoculated plants was not correlated with the extent of varietal susceptibility to bunt. Some lines with resistance, namely those with Bt5 (1.6% infection), Bt8 (0.6%) and Bt10 (0.0%), suffered significantly greater frost kill in the young plant stage as the result of bunt inoculation. By contrast, the Bt7 line had excellent frost resistance and winter hardiness but suffered the greatest extent of bunt infection, whereas the Bt6 line had good frost resistance and good bunt resistance.

Introduction

Hungary is characterised by the extreme weather conditions associated with a continental climate. Low temperature stress is important in reducing the winter survival of cereals. In addition to genetic and environmental factors (including cultivation techniques), the overwintering of winter cereals is also influenced by biotic stress factors, such as plant diseases.

Common bunt, caused by the pathogens *Tilletia foetida* and *T. caries* (Ubrizsy, 1965), occurs in all the wheat-growing regions of the world (Moesz, 1950; Hoffmann & Metzger, 1976; Krivchenko, 1984; Jonsson, 1992; Gaudet et al., 1993; Chauhan et al., 1994). In the past, severe losses due to bunt were recorded in Hungary (Hegyi, 1911), but the consistent application of seed dressings has reduced the impact of bunt in Hungarian wheatfields in recent decades (Kükedi, 1985). *T. foetida* is the most widespread species in Hungary (Kovács, 1955; Podhradszky, 1962).

In addition to yield and quality losses, bunt may also reduce the frost resistance of wheat (Kovács, 1955; Podhradszky, 1962; Babayants, 1988; Zhivotkov, 1989). In order to avoid damage, protective measures must be taken, primarily through seed dressing (Keener et al., 1995), but also by improving genetic resistance (Krivchenko, 1984; Jonsson, 1992; Gaudet et al., 1993). Climatic factors have a considerable influence on the appearance and spread of the pathogen (Podhradszky, 1962; Gaudet & Puchalski, 1989; Johnson, 1992; Gaudet et al., 1993). Since severe natural infection does not occur every year, the establishment and maintenance of artificially inoculated nurseries is necessary if breeding is to be successful (Szunics & Szunics, 1990).

In recent decades wide-ranging, successful research has been carried out in Hungary and abroad...
on the genetic background of frost resistance in cultivated wheat varieties (Law & Jenkins, 1970; Sutka, 1981; Veisz & Sutka, 1989; Limin & Fowler, 1989; Thomashow, 1990; Hayes et al., 1993) and on correlations between the development of frost resistance, the hardening process and environmental factors such as light, temperature, precipitation quantity, and duration of cold hardening (Andrews, 1958; Chen & Gusta, 1978; Steponkus, 1978; De Noma et al., 1989; Kolar et al., 1991). Depending on the preliminary growth and hardening conditions the results of freezing are greatly influenced by the duration and intensity of the cold effect, the extent of freezing and thawing, the dynamics of cooling and the moisture content of the plant tissues (Pomeroy et al., 1975; Chen & Gusta, 1978; De Noma et al., 1989).

Similar environmental conditions are required for the development of bunt infection and for optimum hardening in wheat. It is therefore useful to know how the plant will respond to winter frosts in the presence of common bunt. Apart from a few practical observations very little information is available on the extent to which bunt reduces the overwintering ability of winter wheat varieties and on the mechanism by which this effect is exerted.

The present study deals with the joint effects of common bunt and low freezing temperature on the rate of survival of wheat lines with different degrees of frost resistance and containing bunt resistance genes Bt1 to Bt10.

Materials and methods

The experiments were carried out in an artificially inoculated nursery (bunt resistance, determination of winter hardiness) and the phytotron (frost resistance testing) of the Agricultural Research Institute of the Hungarian Academy of Sciences. Ten wheat lines with various degrees of frost resistance and winter hardiness were assessed. These contained individual genes for resistance to bunt (from Bt1 to Bt10, see McIntosh et al., 1998), and were obtained from Prof Warren Kronstad, Oregon State University, Corvallis, OR, USA.

**Field tests with artificial bunt inoculation (1991–1997)**

The Bt lines were inoculated each year with 0.5 g/1000 seeds of a dry spore mixture obtained from bunt-infected ears collected from commercial wheat fields and experimental nurseries in Hungary. When the weather became favourable for bunt development, generally in late October or early November, 250 seeds were sown per line per plot in 3-row plots measuring 1 m in length with an area of 0.5 m² with three replications on a fellowed, well-prepared area given 80 kg/ha N, 80 kg/ha P, 80 kg/ha K active agents. Susceptibility was determined after harvesting as the number of infected ears as a percentage of the total ears counted.

The following scale (Szunics & Szunics, 1990) was used to determine the resistance of the varieties:

<table>
<thead>
<tr>
<th>Type of resistance</th>
<th>Infected ears (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very resistant</td>
<td>0.0</td>
</tr>
<tr>
<td>Resistant</td>
<td>0.1–5.0</td>
</tr>
<tr>
<td>Moderately resistant</td>
<td>5.1–10.0</td>
</tr>
<tr>
<td>Moderately susceptible</td>
<td>10.1–30.0</td>
</tr>
<tr>
<td>Susceptible</td>
<td>30.1–50.0</td>
</tr>
<tr>
<td>Very susceptible</td>
<td>50.1–100.0</td>
</tr>
</tbody>
</table>

**Freezing tests in the phytotron (1995)**

The frost resistance of Bt lines grown from uninoculated and inoculated seeds was evaluated in the phytotron. Inoculated and uninoculated seeds were sown separately in wooden boxes with internal dimensions of 38 cm × 26 cm × 11 cm. There were 10 rows per box with a different line in each row and 22 seeds per row. There were 4 replications, each box representing one replication. The lines were arranged in the boxes in a randomised complete block design which was the same in the inoculated and uninoculated treatments.

After emergence the plants were raised for six weeks on a climatic programme involving a weekly drop in temperature with a gradual shortening in daylength, similar to the autumn weather conditions in Hungary. (For details of the programme, previously used for frost resistance testing, see Tischner et al., 1997; programme M29).

This was followed by two phases of hardening. The plants were exposed to the first phase in the autumn-winter chamber, where the temperature varied continuously between +3 °C and –3 °C daily for a week, the coldest value being programmed at dawn, as in nature. For the second phase of hardening, the plants were transferred to the frost resistance testing chamber, where they were kept at –4 °C for four days. The