A  n  n  u  a  l   W  h  e  a  t   N  e  w  s  l  e  t  t  e  r             V  o  l .   5  5 .


ITEMS FROM THE RUSSIAN FEDERATION

AGRICULTURAL RESEARCH INSTITUTE OF THE CENTRAL REGION OF NON-CHENOZEM ZONE
143026, Moscow region, Nemchinovka, Kalinina 1, Russian Federation.

Soft wheat hybrids showing no segregation for resistance to leaf rust.


The soft winter wheat cultivar Nemchinovskaya 24 has demonstrated absolute resistance to leaf rust since the time of its release 20 years ago. In order to understand the genetic basis of the resistance, we crossed Nemchinovskaya 24 with tester lines of spring wheat with genes Lr9, Lr24, Lr24 + Sr24, Lr27 + Lr31, Lr28, Lr29, Lr38, and LrTr). The susceptible soft spring wheat Khakasskaya was used as a check.

The F1 hybrids and their parental lines were not susceptible to leaf rust and that the resistance genes of their parental lines appeared to be dominant. The F2 hybrid progeny of the cross ‘Nemchinovskaya 24 / Khakasskaya’ segregated according to a trihybrid pattern, 43 resistant plants : 21 susceptible plants (Table 1).

We found the action of one main and two complementary inhibiting genes. F1 hybrids between stocks with Lr24, Lr27 + Lr31, Lr28, and Lr29 with Nemchinovskaya 24 segregated according to a dihybrid pattern (15 resistant: 1 susceptible). The F2 progenies from lines with Lr9, Lr24 + Sr24, Lr38, and LrTr are interesting because no plants were susceptible to leaf rust. All the plants are

<table>
<thead>
<tr>
<th>Cross</th>
<th>Number of resistant plants</th>
<th>Number of susceptible plants</th>
<th>Ratio of resistant to susceptible plants</th>
<th>χ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>N24 / Khakasskaya</td>
<td>120</td>
<td>64</td>
<td>42 : 22</td>
<td>0.324</td>
</tr>
<tr>
<td>N24 / Lr9</td>
<td>127</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Lr24 / N24</td>
<td>80</td>
<td>6</td>
<td>13.3 : 1</td>
<td>0.078</td>
</tr>
<tr>
<td>N24 / Lr24+Sr24</td>
<td>157</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Lr27+Lr31 / N24</td>
<td>122</td>
<td>7</td>
<td>17.4 : 1</td>
<td>0.149</td>
</tr>
<tr>
<td>Lr28 / N24</td>
<td>138</td>
<td>9</td>
<td>15.3 : 1</td>
<td>0.004</td>
</tr>
<tr>
<td>Lr29 / N24</td>
<td>61</td>
<td>4</td>
<td>15.3 : 1</td>
<td>0.001</td>
</tr>
<tr>
<td>N24 / Lr38</td>
<td>171</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>N24 / LrTr</td>
<td>181</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Critical χ² = 3.84.

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173
resistant. The experimental evidence indicates that the resistance genes of the parental forms are located on homologous chromosomes, but we have not identified them or determined their allelism. The question that remains unanswered is why the $F_2$ hybrid population of ‘$Lr24$ / Nemchinovskaya 24’ segregated for resistance to leaf rust, whereas the cross ‘Nemchinovskaya 24 / $Lr24 + Sr24$’ produced no susceptible plants in the $F_2$.

Sieptoria sp. fungi affect all wheats to one extent or another. No fully disease-resistant wheat is known in the world collection. Plants with relative resistance are sensitive to the pathogen at later developmental stages. One example is the Bulgarian winter wheat PI476772 from the Moscow International Science and Technology Center’s collection. The line also is resistant to leaf rust and highly resistant to mildew. Hybrid progenies from crossing this line with Nemchinovskaya 24 also are resistant to leaf rust (% infection in both $F_1$ and $F_2 = 0$), and Septoria develops late on them. According to preliminary data obtained by our laboratory, this Bulgarian wheat has the genotype $Lr10, Lr26,$ and $Lr46$.

Nemchinovskaya 24 soft winter wheat is resistant to leaf rust. It will be necessary to identify the resistance genes. Using hybrid populations, which are not susceptible to leaf rust and not segregating for resistance, in soft wheat selection for resistance to leaf rust can be effective.

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Department of Genetics, Laboratory of Genetics and Cytology, 7 Toulaiikov St., Saratov, 410010, Russian Federation.

**The evaluation of spring bread wheat cultivars, NILs, and promising lines to leaf, stem, and stripe rusts in 2008.**


In 2008, during the vegetative period of spring bread wheat, leaf, stem, and stripe rusts epidemics were observed and evaluated. The severities of these diseases were different. Leaf rust was estimated as moderate. Atem and stripe rusts were observed as weak. Evaluation of a set NILs carrying $Lr$ genes show that the severity of the leaf rust epidemic on susceptible cultivars was 50–55%. Highly resistant lines (IT = 0;1) had the genes $Lr9, Lr28,$ and $Lr29$ and gene combinations $Lr9+Lr19, Lr19+Lr24, Lr19+Lr25,$ and $Lr19+Lr26$. Interestingly, genes $Lr28$ and $Lr29$ showed an IT = 0;1, but several years ago (2000) these genes had an IT = 3. Hence, within eight years sharp changes in the set of pathotypes has taken place, enabling $Lr28$ and $Lr29$ to be highly effective.

The evaluation of promising spring bread wheat lines to stem and stripe rusts was made in the southwest part of the Saratov region. An IT = 0 in the NILs and promising lines had the following combinations of $Sr$ genes: $Sr24+Sr25$ and $Sr25+Sr31$. The majority of spring bread wheat sowings in this zone include the cultivars L503, L505, Belyanka and Dobrynya. The cultivars L503, L505, and Dobrynya had ITs = 0; Belyanka had an IT of 3. Resistance to stripe rust in the L503, L505, and Dobrynya controlled by an unidentified $Yr$ gene(s). This $Yr$ gene(s) was transmitted from the above-mentioned cultivars into the promising lines.

**Agronomic performance of multilinear mixes on the basis of spring bread wheat cultivar Dobrynya.**

S.N. Sibikeev, I.N. Cherneva, and A.E. Druzhin.

The perceived advantages of mixtures over their components in monoculture include larger yields, more stable performance, and improved and more durable resistance to diseases. In 2008, we investigated multilinear mixes on the basis of cultivar Dobrynya. These mixes include four components: Dobrynya, Dobrynya $Lr19+Lr9$, Dobrynya $Lr19+Lr24$, Dobrynya $Lr19+Lr25$, and all components in equal parts. We also used mixtures of the first (prepared in 2008) and second years (after cultivation in 2007). The control used all lines and Dobrynya. We looked at the agronomical traits heading date, plant height, resistance to lodging, 1,000-kernel weight, and grain productivity. For heading date, the
multilinear mixes did not differ from lines or cultivar. For plant height, the component lines did not significantly differ among themselves however, Dobrynja Lr19+Lr24 was greater on average. Multilinear mixes did not significantly differ for plant height from the component average, but mixes of the first and the second year cultivation were higher than the components, averaging 5–6 cm. For lodging resistance, we observed that the first-year mix was more resistant than the component lines, but the second-year mix was not significantly lower. For 1,000-kernel weight, significance was not observed, but the first-year mix was higher than that of the second-year mix and lower than component average. For grain productivity, the first-year mix was not significantly different from component average, although an increase in productivity was 10%. The second-year mix was significantly higher than the component average at 26%. We are now analyzing pustule number for leaf rust on a susceptible component mixture, spike productivity, flour quality of components and mixes.

**Influence on disease resistance of translocations from Thinopyrum intermedium; Th. elongatum; Secale cereale; T. turgidum subsp. durum, dicoccoides, and dicoccum; and T. timopheevii subsp. timopheevii in spring bread wheat lines.**


For four years (2005–08), spring bread wheat lines carrying translocations from *Th. intermedium; Th. elongatum; S. cereale; T. turgidum* subsp. *durum, dicoccoides, and dicoccum; and T. timopheevii* subsp. *timopheevii* were investigated for resistance to some diseases (Table 1). A combination of alien chromatin from several species in one genotype significantly improves disease resistance in these lines. The spring bread wheat line L2772, with chromatin from both *Th. intermedium* and *T. turgidum* subsp. *durum* has race-specific resistance to a loose smut and resistance to leaf rust, powdery mildew, and common bunt in comparison with the parental lines. Similar results have been obtained for line L2780, which combines chromatin from *Th. elongatum* and *T. timopheevii* subsp. *timopheevii* was nearly resistant to loose smut, leaf rust, and powdery mildew and common bunt. Multilinear mixes did not significantly differ from lines or cultivar. For plant height, the component lines did not significantly differ among themselves however, Dobrynja Lr19+Lr24 was greater on average. Multilinear mixes did not significantly differ for plant height from the component average, but mixes of the first and the second year cultivation were higher than the components, averaging 5–6 cm. For lodging resistance, we observed that the first-year mix was more resistant than the component lines, but the second-year mix was not significantly lower. For 1,000-kernel weight, significance was not observed, but the first-year mix was higher than that of the second-year mix and lower than component average. For grain productivity, the first-year mix was not significantly different from component average, although an increase in productivity was 10%. The second-year mix was significantly higher than the component average at 26%. We are now analyzing pustule number for leaf rust on a susceptible component mixture, spike productivity, flour quality of components and mixes.

**Table 1. The infection type (IT) of spring bread wheat lines to leaf rust, powdery mildew, loose smut, and common bunt.**

<table>
<thead>
<tr>
<th>Line</th>
<th>Pedigree</th>
<th>Leaf rust</th>
<th>Powdery mildew</th>
<th>Loose smut</th>
<th>Common bunt</th>
</tr>
</thead>
<tbody>
<tr>
<td>L.3065</td>
<td>Saratovskaya 55/<em>Th. elongatum</em>&lt;sup&gt;3&lt;/sup&gt;/Saratovskaya 29</td>
<td>3</td>
<td>2</td>
<td>8.8</td>
<td>26.3</td>
</tr>
<tr>
<td>L.215</td>
<td>Saratovskaya 55&lt;sup&gt;4&lt;/sup&gt;/<em>T. turgidum subsp. dicoccoides</em></td>
<td>0</td>
<td>0</td>
<td>28.8</td>
<td>21.5</td>
</tr>
<tr>
<td>L.196</td>
<td>S58/<em>T. turgidum subsp. dicoccoides</em>&lt;sup&gt;3&lt;/sup&gt;/S58</td>
<td>1</td>
<td>2</td>
<td>70.0</td>
<td>66.8</td>
</tr>
<tr>
<td>L.2780</td>
<td>CI-12633/I504</td>
<td>0</td>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>L.2816</td>
<td>I528/Saratov. golden</td>
<td>0</td>
<td>0</td>
<td>6.3</td>
<td>4.7</td>
</tr>
<tr>
<td>L.2358</td>
<td>L401/<em>T. turgidum subsp. dicoccum</em>&lt;sup&gt;4&lt;/sup&gt;/L401/S55/L2033/S60/Prohorovka</td>
<td>0</td>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>L.3630</td>
<td>L2040/Prohorovka</td>
<td>2</td>
<td>1</td>
<td>5.5</td>
<td>4.5</td>
</tr>
<tr>
<td>L.805</td>
<td>L2040/Lut.13-80</td>
<td>0</td>
<td>2</td>
<td>4.8</td>
<td>8.5</td>
</tr>
<tr>
<td>L.2772</td>
<td>L164/L.222</td>
<td>0</td>
<td>1</td>
<td>13.3</td>
<td>35.8</td>
</tr>
</tbody>
</table>
had a low level of an infection by common bunt. Resistance to a complex of diseases was shown line L2358, with *T. turgidum* subsp. *dicoccum* and *Th. elongatum* chromatin.

Of particular interest is L3065, which contains a chromatin from *Th. elongatum*. This line shows race-specific resistance to loose smut and pathotypes of common bunt but is susceptible to leaf rust and powdery mildew. Line L215, which was produced from crosses with *T. turgidum* subsp. *dicoccooides*, has resistance to leaf rust and powdery mildew, moderate resistance to common bunt, and race-specific resistance to loose smut. Small lesions from leaf rust and common bunt were shown line L196, which has chromatin from *T. turgidum* subsp. *dicoccum*, but is susceptible to the pathotypes of loose smut investigated here.

A combination in one genotype of alien genes with own genes from *T. aestivum* gives a positive effect. Thus, lines L3630 and L805, which include line L2040 in their pedigree (contains chromatin from *T. turgidum* subsp. *durum*), have shown a high level of resistance to the pathotypes of loose smut investigated, and they also have tolerance to leaf rust and powdery mildew.

These data show that combining several alien genes (chromatins) in one bread wheat genotype can produce lines with complex diseases resistance or lower the degree of damage.

**Effects of an Lr26 translocation on grain productivity and grain protein content in spring bread wheat.**

S.N. Sibikeev, O.V. Krupnova (Laboratory for Evaluation of Grain Quality), S.A. Voronina, V.A. Krupnov, and A.E. Druzhin.

The first fertile hybrids between wheat and rye were received in Saratov in 1918. However, the using the desirable genetic material from the rye gene pool for improvement of spring wheat has been limited by undesirable linkages in the rye translocations, in particular with genes that decrease grain quality. At ARISER, based on the best Saratov-bred spring bread wheat cultivars, a set of NILs with the Lr26 translocation were produced. The donors of the translocation with Lr26 were Genaro 81 and an NIL of Thatcher. The Saratov population of leaf rust included virulent pathotypes to Lr26 and Lr19. The combination of translocations with Lr26 from *S. cereale* and Lr19 from *Th. elongatum* is effective against the Saratov population of *P. triticina*. Under leaf rust epidemics in 2004 and 2005, this combination of translocations positively effected grain productivity (t/ha), grain protein content (%), and grain protein yield (t/ha). In the hard drought conditions of 2007, grain productivity and grain protein content of lines containing a combination of Lr19 + Lr26 did not significantly differ from the checks with only one translocation (Lr19 or Lr26). The 6-year average of lines L706-02 and L785-02 (L503*5/Tc Lr26) compared with L503 were 0.20 and 0.41% for grain protein content and 12.9 and 6.1% for grain protein yield, respectively.

**Effect of a translocation from Thinopyrum intermedium on preharvest-sprouting resistance in wheat lines.**


Preharvest-sprouting resistance is important for the production of grain from spring bread wheat in the Volga Region. The cultivars L503, L505, Dobrynya (= L1089), and lines L 2032 and L 583) spring bread wheat have a translocation from *Th. elongatum* with Lr19 and were resistant to preharvest sprouting (germination index from 0.12 to 0.32), but these cultivars and lines were susceptible to the leaf rust population in Saratov.

The lines L400 and M6R, which have a translocation or whole chromosome from *Th. intermedium*, are resistant to the pathogen but highly susceptible to preharvest sprouting (germination index from 0.87 to 0.95). The F₁ – F₇ RIL populations with resistance to leaf rust and high grain yield and good quality flour is from a crosses between cultivars L503, L505, Dobrynya, and lines L 2032 and L 583 (all red-grained) with two lines L400 and M6R (susceptible to pre-harvest spouting) were studied. A total of 41 lines and parents were grown in the field from 2003 to 2006. Each line was represented by a plot of seven 7-m rows with 0.15 m interrow spacing in a randomized complete-block design with four replications. Among the selected lines were 22 red-grained (germination index from 0.31 to 0.93) and 19 white-grained
Preharvest-sprouting resistance of the red-grained sibs and three NIL pairs were significant higher than that of the white-grained lines. L204 (red grained) and L205 (white grained) NILs were identical and equally susceptible to preharvest spouting. The germination index of lines ‘BC1F6Л2032*2/M6R’ was significantly higher than that of L2032. L400 is a 400S sib line that does not have the Th. intermedium translocation. The preharvest-sprouting resistance of 400S was significantly higher than that of L400 only in 2003.

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Using of cultivar mixtures of soft spring wheat for improving technological qualities of grain in the Russian Far East.

I.M. Shindin and S.V. Phirstov.

Developing cultivars with high technological and baking qualities is the most complicated problem in soft spring wheat selection in the Russian Far East (Shindin and Cherpak 2005). To resolve that problem, we are interested in mixtures consisting of cultivars that are remarkable for their technological and baking qualities. Some scientists determined that cultivar mixtures, as complicated populations, are resistant to abiotic and biotic stresses and have more stable yields and grain quality than homologues cultivars under changeable weather conditions (Sekun 1951; Martynyuk 1964; Kuzmin 1966; Vedrov et.al 1998).

We used the cultivars Khabarovchanka, Zaryanka, and Lira 98, grown in the Far Eastern region, for our mixture. Lira 98 is most valuable for food grain quality among the three cultivars. Lira 98 is used to improve the technological and baking features of Khabarovchanka and Zaryanka, which are less valuable but highly productive and resistant to lodging and disease. A two-cultivar mixture (Khabarovchanka + Lira 98) was 50:50, and the three-cultivar mixture (Khabarovchanka + Lira 98 + Zaryanka) was 33:33:33%.

A comparative analysis of the cultivars and their mixtures showed that Lira 98 and two mixtures turned out to be the best ones by their technological and baking qualities (Table 1). According to the State Standards of the Russian Federation (GOST RF), grain from all the cultivars conforms to the standard of an appreciable sort of wheat. Also important is that the cultivar mixtures yield similar to the initial cultivars in the years of drought, and 10–15% higher in the years of humid weather.

<table>
<thead>
<tr>
<th>Cultivars and mixtures</th>
<th>Grain vitreousness (%)</th>
<th>Dough elasticity (alveograph, mm)</th>
<th>Elasticity and stretching ratio (alveograph units)</th>
<th>Flour strength (alveograph units)</th>
<th>Gluten content (%)</th>
<th>Bread output from 100 g of flour (mL)</th>
<th>Baking quality (mark)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Khabarovchanka</td>
<td>56</td>
<td>115</td>
<td>1.9</td>
<td>311</td>
<td>32.4</td>
<td>871</td>
<td>3.6</td>
</tr>
<tr>
<td>Zaryanka</td>
<td>55</td>
<td>134</td>
<td>2.4</td>
<td>331</td>
<td>33.5</td>
<td>950</td>
<td>3.8</td>
</tr>
<tr>
<td>Lira 98</td>
<td>77</td>
<td>121</td>
<td>1.7</td>
<td>497</td>
<td>37.0</td>
<td>1,010</td>
<td>4.2</td>
</tr>
<tr>
<td>Khabarovchanka + Lira 98</td>
<td>66</td>
<td>110</td>
<td>1.2</td>
<td>469</td>
<td>32.4</td>
<td>1,040</td>
<td>4.2</td>
</tr>
<tr>
<td>Khabarovchanka + Lira 98 + Zaryanka</td>
<td>61</td>
<td>106</td>
<td>1.0</td>
<td>438</td>
<td>35.2</td>
<td>1,000</td>
<td>4.0</td>
</tr>
</tbody>
</table>
**Races of Puccinia graminis f. sp. tritici in the Russian Federation in 2007.**

S.N. Lekomtseva, V.T. Volkova, L.G. Zaitseva, and E.S. Skolotneva.

The wheat stem rust pathogen, having an extremely high ability to evolve new, virulent phenotypes (such as Ug99), is one of the most important monitored pests that needs annual control in cereal-growing countries. Last year in the Russian Federation, the survival strategy of *P. graminis* f.sp. *tritici* was to emphasize barberry and wild grasses being limited on the wheat cultivars (Lekomtseva et al. 2007).

In the summer of 2007, unfavorable conditions controlled the spread of stem rust in the European part of the Russian Federation (Central Region and Northern Caucasus) and Western Siberia. The average temperature was about 22°C with 45% relative humidity in June–July. Local wheat cultivars were quite resistant to stem rust under these climactic conditions.

Barberry was heavily infected by *P. graminis* f.sp. *tritici* in all these regions during May and the first week of June. Furthermore, in early autumn, wild grasses (*Elytrigia, Phleum,* and *Festuca*) were severely damaged by wheat stem rust. The spores of *P. graminis* f.sp. *tritici* from barberry and wild grasses were collected and multiplied on the susceptible wheat cultivar Khakasskaya. Race identification of 32 monouredinial isolates of *P. graminis* f.sp. *tritici* was carried out using the standard technique of infection of 20 wheat lines, which were supplied by the USDA–ARS Cereal Disease Laboratory, St. Paul, Minnesota, USA, in 2005.

In Pgt nomenclature (Roelfs and Martens 1988), six races of *P. graminis* f.sp. *tritici* were revealed among the geographical samples (Table 1), and all phenotypes were combined in the single, highly virulent, Stackman’s race 15. Race TKNTF (15) dominated in the different regions of the Russian Federation with a frequency of 75% in populations of the fungus.

The race composition of *P. graminis* f. sp. *tritici* on barberry in Northern Caucasus was significantly different from that in Central Russia and Western Siberia. Only two races were found in Central Russia with TKNTF (15) dominating. TKNTF also was prevalent in Western Siberia. No race was dominant of the five virulent phenotypes identified in the Northern Caucasus region, although one of these races was TKNTF (15). Intensive sexual process provides high variability of race composition on barberry in the mountains of Northern Caucasus. This determines

### Table 1. Races of *Puccinia graminis* f.sp. *tritici* in some regions of the Russian Federation in 2007.

<table>
<thead>
<tr>
<th>Race</th>
<th>Area</th>
<th>Plant host</th>
<th>Number of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>TKNTF</td>
<td>Central area</td>
<td>barberry</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Northern Caucasus</td>
<td>barberry</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Central area</td>
<td>couch grass</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Central area</td>
<td>fescue</td>
<td>1</td>
</tr>
<tr>
<td>TKSTF</td>
<td>Central area</td>
<td>barberry</td>
<td>1</td>
</tr>
<tr>
<td>TTSTF</td>
<td>Northern Caucasus</td>
<td>barberry</td>
<td>1</td>
</tr>
<tr>
<td>PKNTF</td>
<td>Northern Caucasus</td>
<td>barberry</td>
<td>1</td>
</tr>
<tr>
<td>TTNTF</td>
<td>Northern Caucasus</td>
<td>barberry</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Western Siberia</td>
<td>barberry</td>
<td>1</td>
</tr>
<tr>
<td>TKNRF</td>
<td>Central area</td>
<td>couch grass</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Central area</td>
<td>timothy grass</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>32</td>
</tr>
</tbody>
</table>
this region as the most infective source of different virulent phenotypes of stem rust pathogen for cereal grasses. In Central Russia, race TKNTF dominated on coach grass (Elytrigia), timothy grass (Phleum), and fescue (Festuca) in addition to barberry. The virulent phenotype TTKS (Ug99) was not fixed in 2007 and was not found before (Lekomtseva et al. 2004, 2007). Some of the Sr genes of wheat, Sr9b, Sr13, Sr24, and Sr31 were resistant to all stem rust isolates in 2007 (Table 2). Gene Sr11 was susceptible during 2001–05 but is now resistant. All these genes are recommended for plant-breeding programs to use against the wheat stem rust pathogen in the Russian Federation.

Acknowledgment. This work is supported by the Russian Foundation of Basic Researches (project No. 08-04-00492).

References.

Table 2. Virulence of isolates of Puccinia graminis f.sp. tritici to Sr lines of wheat 2007 in the Russian Federation (%).

<table>
<thead>
<tr>
<th>Gene</th>
<th>%</th>
<th>Gene</th>
<th>%</th>
<th>Gene</th>
<th>%</th>
<th>Gene</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sr5</td>
<td>100.0</td>
<td>Sr9b</td>
<td>9.3</td>
<td>Sr11</td>
<td>21.0</td>
<td>Sr31</td>
<td>0.0</td>
</tr>
<tr>
<td>Sr6</td>
<td>100.0</td>
<td>Sr9c</td>
<td>100.0</td>
<td>Sr13</td>
<td>0.0</td>
<td>Sr36</td>
<td>100.0</td>
</tr>
<tr>
<td>Sr7b</td>
<td>100.0</td>
<td>Sr9d</td>
<td>100.0</td>
<td>Sr21</td>
<td>97.0</td>
<td>Sr38</td>
<td>100.0</td>
</tr>
<tr>
<td>Sr8a</td>
<td>100.0</td>
<td>Sr9e</td>
<td>100.0</td>
<td>Sr24</td>
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</table>

Encapsulating winter wheat seed.

N.V. Poukhalskaya, V.A. Budkov, and Dm. Koluntaev.

Encapsulating seed creates a macronutrient environment for starting seed. The surface of seed adsorbs NPK and this is more effective than adding to in soil nearby. However, the negative effect of this method is an increase in the osmotic concentration of solution and the high sensitivity of seedlings.

Materials and methods. Seedlings of winter wheat were grown for 10 days at 21°C in plastic cups. Before germination, the seeds were encapsulated with four different solutions: 1 – H₂O, 2 – a 1% soluble, complex fertilizer (NPK), 3 – a 2.5% soluble, complex fertilizer (NPK), and 4 – a 5% soluble, complex fertilizer (NPK).

Results and discussion. The swelling dynamics of seed and the change in the seed humidity in first four days of growth depended upon the concentration of the treatment solution. High concentrations of solution diminished the speed of germination substantially (Fig. 1). The first negative effect observed on the encapsulated seeds was a decline in germination (Fig. 2, p. 180). Increasing the concentration of the treatment solution caused a decline in seed germination (black) and multiplied the number of seed that perished after primary germination (white) (Fig. 2, p. 180).
For the first seven days, a plant is nourished from the seed. For this reason, after four days H$_2$O-treated seedlings overtake encapsulated treated seeds by more than 20%. But at nine days after germination, encapsulated seeds begin to overtake the control plants in proportion to the treatment (Fig. 3).

Using seed encapsulation under soil stress conditions (at a high concentration of Al ions in the soil solution) reduces the negative effect of the high concentration of NPK in the treated seeds. At an increase in the soil solution of the aluminum ions in an encapsulation solution renders a protective effect, causing formation of Al complexes and an increase in seedling growth.

**Fig. 2.** Germination (%) and the number of dead seedlings after the first five days for different concentrations of encapsulation solutions (Control is H$_2$O only).

**Fig. 3.** Length of seedlings (mm) after four and nine days growth for different concentrations of encapsulation solutions (Control is H$_2$O only).

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**Estimating different kinds and lines of spring bread wheat for total resistance to fungus diseases.**


Since 2005, we estimated the resistance of material for loose smut, powdery mildew, and leaf and stem rust in the city of Saratov, Russian Federation. The total resistance to all investigated pathotypes of loose smut have shown that the cultivars Zhigulevskaya and Saratovskaya 70 and lines L658-01 and L2040 are most resistant. The cultivars Lutescens 62, Dobrinja, L503, and L504 were susceptible to all the investigated patotypes. Other cultivars showed race-specific resistance.

A pedigree analysis of the cultivars with sources of resistance to any pathotype of loose smut included *T. turgidum* subsps. *durum*, *dicoecum*, and *turgidum*, *T. timopheevi* subsp. *timopheevii*, and *Elytrigia intermedia* and also the cultivars Krimka (a local winter wheat cultivar from Ukraine), Ostka Halisijskaja (a spring bread wheat from Poland),
Selivanovskij Rusak (a local spring bread wheat cultivar from the Volga region), and Beloturka (a local durum cultivar from the Volga region).

Resistance also was studied to leaf and stem rust and powdery mildew in spring bread wheat. Lines selected carrying alien genes that would ensure total resistance to leaf and stem rust and powdery mildew were L2166 and L784/03; for resistance to leaf and stem rust was L2075; for resistance to leaf rust and powdery mildew were Мульти 6R, L2505, L1059, L484/03; and L487/03; for resistance to leaf rust were L1078, L2608, and L2870; and for resistance to powdery mildew was L2032. The donors of resistance to these diseases are Ae. speltoides, S. cereale, Th. intermedium, and T. turgidum subsps. durum and dicoccoides.

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Novel antimicrobial peptides from seeds of Triticum kiharae and Leymus arenarius.

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To protect themselves against pathogens, plants produce a wide array of antimicrobial proteins and peptides (AMPs), some of which are synthesized constitutively, whereas others are induced upon challenge with pathogenic microorganisms (Selitrennikoff 2001; Garcia-Olmedo et al. 2001). Each plant genome encodes hundreds AMPs (Manners 2007). Such biodiversity ensures efficient defense against numerous invading and constantly evolving microorganisms. Most plant AMPs belong to cysteine-rich peptides and contain an even number of cysteine residues, all of which are involved in the formation of intrachain disulphide bridges providing their molecules with high structural stability. Based on cysteine spacing motifs and three-dimensional structures several families of antimicrobial peptides have been discriminated in plants (Broekaert 1997). Hevein-type peptides show structural similarity to the 43-amino-acid residue chitin-binding peptide isolated from the rubber tree Hevea brasiliensis L. (Van Parijs et al. 1991) and comprise the single-hevein-domain subfamily in a large group of chitin-binding proteins implicated in plant defense (Raikhel and Lee 1993). Despite sequence similarity, hevein-type AMPs differ in the number of disulphide bonds. Most of them possess 8 cysteine residues forming 4 disulphide bonds and in this respect are close to the chitin-binding domains of class I/IV chitinases (Beintema 1994). Truncated variants with only six cysteine residues also occur (Broekaert et al. 1992). AMPs are regarded as promising agents for plant transformation and production of resistant crops, therefore the search for new, highly potent AMPs is a rapidly developing area of research.

We focused on AMPs from seeds of two Poaceae species, Leymus arenarius and Triticum kiharae. In contrast to T. kiharae, L. arenarius grows in a narrow shore region of the White Sea at high soil salinity. We show that both species possess highly homologous hevein-type peptides of unusual structure, which effectively inhibits growth of a wide range of plant pathogens at micromolar concentrations.

Materials and methods. The species used in this study were T. kiharae Dorof. et Migush. and L. arenarius; the fungi and bacteria Fusarium solani VKM F-142, F. verticillioides VKM F-670, F. oxysporum TSA-4, Botrytis cinerea VKM F-85, Neurospora crassa VKM F-184, Pseudomonas syringae VKM B-1546, Clavibacter michiganense subsp. michiganense VKM Ac-1144, and Erwinia carotovora subsp. carotovora VKM B-1247 were obtained from the All-Russian Collection of Microorganisms.

Flour was extracted with 10% acetic acid for 1 h at room temperature and desalted on an Aquapore RP300 column. Freeze-dried acidic extract was subjected to chromatography on Heparin Sepharose. Proteins and peptides were
eluted with a stepwise NaCl gradient. The 100-mM NaCl fraction was collected, desalted as described above, and separated on a Superdex Peptide HR 10/30 column (Amersham, Pharmacia, Biotech, Uppsala, Sweden). Proteins and peptides were eluted with 0.05% TFA, containing 5% acetonitrile, at a flow rate of 250 μL/min and monitored by absorbance at 214 nm. The peptide fraction was further separated by RP-HPLC on a Vydac C18 column (4.6 x 250 mm, particle size 5 μm) with a linear acetonitrile gradient (10-50%) for 1 h at a flow rate of 1 mL/min and 40°C. Peptides were detected at 214 nm. Mass spectra were acquired on a model Reflex III mass spectrometer (Bruker Daltonics, Bremen, Germany). N-terminal amino acid sequences were determined by automated Edman degradation on a model 492 Procise sequencer (Applied Biosystems) according to the manufacturer’s protocol.

The antifungal activity of the peptides was tested against several fungi using microtiter-plate assays. Wells were filled with 10 μL of two-fold serial dilutions of the peptide and mixed with 90 μL half-strength potato-glucose broth containing approximately 10⁴ spores/mL. The inhibition of spore germination was evaluated by measuring the absorbance at 620 nm. The antibacterial activity of peptides was assayed against several Gram-positive and Gram-negative bacteria using radial diffusion assay. Petri dishes with LB agar were seeded with test bacteria. The peptide solutions (50 μL) were applied to the wells (5 mm in diameter) punched into the agar, and the Petri dishes were incubated at room temperature for 24-48 h. Antibacterial activity was evaluated by the size of the inhibition zone formed around the wells with the peptide solution. The antibiotic claforan and sterile water were used as controls.

**Results and discussion.** For the isolation of AMPs from *T. kiharae* and *L. arenarius*, we followed the procedure developed for the isolation of *T. kiharae* defensins (Egorov et al. 2005; Odintsova et al. 2006), which included acidic extraction of flour followed by subsequent separation of the protein-peptide extract by a combination of different types of HPLC (affinity, size-exclusion and reversed-phase). As a result, two novel peptides named WAMP and LAMP were isolated from seeds of *T. kiharae* and *L. arenarius*, respectively. The measured monoisotopic molecular masses of the peptides were 44,31 and 4,444 Da for WAMP and LAMP, respectively. Their amino acid sequences were determined by automated Edman degradation after reduction and alkylation.

Considerable sequence similarity with hevein and homologous peptides was revealed providing evidence that both peptides belong to hevein-type AMPs. However, in contrast to hevein, they possess ten cysteine residues and, thus, may be classified as 10-Cys hevein-like peptides. Only two 10-Cys hevein-like peptides have been described so far, isolated from the bark of the trees *Eucommia ulmoides* Oliv. (Huang et al. 2002) and *Euonymus europaeus* L. (Van den Bergh et al. 2002). Despite similarity in the number of cysteine residues, the cysteine motif in WAMP and LAMP differs remarkably from that of their 10-Cys homologues indicating that isolated peptides belong to a new subfamily of hevein-type peptides. Striking similarity with hevein-type domains of cereal class-I chitinases both in amino acid sequences and cysteine patterns was noticed.

Thiol-specific alkylation of unreduced native WAMP and LAMP peptides did not result in molecular mass changes pointing to the involvement of all 10 SH-groups in the formation of 5 disulphide bridges in each peptide. The measured molecular masses of the peptides were in good agreement with calculated values indicating the absence of post-translational modifications except disulphide bridges. Based on sequence similarity with hevein, for which the cysteine connectivities are known, disulphide bridges in WAMP were predicted as follows: C⁴–C¹⁹, C¹–C²⁸, C¹⁹–C⁴², C⁷⁷–C⁴¹. An additional fifth disulphide bond is likely to be formed between C¹⁶ and C⁴⁴. Because chitin is the main component of fungal cell walls and exoskeleton of insects, chitin-binding activity is assumed to be indicative of the ability of polypeptides to inhibit growth of phytopathogenic fungi or pests. The chitin-binding properties of WAMP and LAMP peptides were assayed in vitro. Purified peptides were applied to a chitin column and the bound fraction was eluted with 0.1% TFA. RP-HPLC and mass measurements of unbound and bound fractions showed that both peptides eluted only in the bound fraction thus providing evidence that they bind to chitin. Thus both peptides WAMP and LAMP bind chitin. The inhibitory activity of both peptides towards several pathogens was assayed directly. The results for WAMP are presented in Table 1.

<table>
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<tr>
<th>Fungus</th>
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<tr>
<td><em>Fusarium solani</em></td>
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<tr>
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<td>30</td>
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Testing of the biological activity of the recombinant peptide WAMP against several fungi including deiteromycetes and ascomycetes showed marked inhibition of spore germination at micromolar concentrations with an IC₅₀ ranging from 5 to 30 μM depending on the fungus (Table 1). The highest inhibitory activity was achieved against *F. solani*; the
IC$_{50}$ for this fungus was 5 μM. The WAMP peptide was also tested for inhibition of bacterial growth against both Gram-positive (C. michiganense) and Gram-negative bacteria (P. syringae and E. carotovora); for the Gram-positive bacterium C. michiganense the effect was most pronounced (Table 2). The antifungal activity of WAMP is likely to be associated with its chitin-binding activity, whereas the inhibitory effect on bacteria, which are devoid of chitin, implies the existence of some other mechanism.

In summary, two novel, highly homologous, hevein-type and chitin-binding AMPs, WAMP and LAMP, which share sequence similarity with chitin-binding domains of cereal class-I chitinases, were purified from T. kiharae and L. arenarius seeds. To the best of our knowledge, this is the first report on the occurrence of 10-Cys hevein-type peptides in plant seeds. The cysteine motif in WAMP and LAMP is new and distinct from those of other previously characterized hevein-type AMPs providing evidence that they belong to a new structural type of AMPs. The peptides showed potent antifungal and antibacterial activities at micromolar concentrations and, thus, may be used in genetic transformation of plants to enhance resistance to pathogenic microorganisms.

**References.**


Influence of bacterial lipopolysaccharide on the morphogenetic and morphometric parameters of cultivation of wheat somatic callus.

Yu.V. Lobachev and O.V. Tkachenko (Vavilov Saratov State Agrarian University) and L.Yu. Matora, G.L. Burygin, and N.V. Evseeva (Institute of Biochemistry and Physiology of Plants and Microorganisms).

A major challenge in cultivating plants is ensuring that the cells preserve their morphogenetic potential. The capacity of plant explants for morphogenesis depends on the plants’ genotypic peculiarities and on nutrient-medium composition and culture conditions (Yezhova 2003). In addition, plant-associated methylobacteria promote accelerated seed germination and the further seedling growth in vivo (Fall 1996), and they also stimulate plant growth and morphogenesis in vitro (Kalyayeva et al. 2001). N2−fixing bacteria promote plant growth only in vivo (Steenhoudt and Vanderleyden 2000). Inoculation in vitro of plants with these bacteria is technically difficult (Korzhenevskaya 1990). In this context, it is important to treat explants not with a bacterial suspension but with bacterial-cell components that determine the plant-bacterium interaction. The outer-membrane lipopolysaccharide (LPS) of N2−fixing bacteria of the genus Azospirillum is an active bacterial component that not only determines contact bacterium-plant root interactions (Fedonenko et al. 2001) but also is involved in the processes inducing plant responses to these interactions (Matora et al. 1995). The aim of this work was to examine the influence of LPS on the morphometric and morphogenetic parameters of cultivation of wheat somatic callus.

Immature embryos of two model near-isogenic lines (genetic background of cultivar Saratovsraya 29), differing in the Rht-B1c alleles were placed on a callus-initiation nutrient medium that contained 2.5, 5, and 10 mg/l LPS. The resulting morphogenic callus were transferred to a regeneration medium with the same LPS content. A study of callus initiation and the cultures’ regeneration ability showed that the LPS at the concentrations used did not have a significant effect on the formation of morphogenic callus or on the ability of the Rht-B1c gene to increase this parameter, found by us previously (Tkachenko and Lobachev 2008). LPS slightly increased the mass of morphogenic and nonmorphogenic callus. The regeneration ability of the callus and the dynamics of formation of regenerated plants did not change in the presence of the LPS. In summary, the LPS at 2.5, 5, and 10 mg/l did not have a significant effect on the morphogenetic parameters of in vitro cultivation of wheat somatic cells. A search further for effective concentrations or for a method of introducing LPS into a nutrient medium for cultivation of wheat somatic embryos will be necessary.

References.


During the past several decades, plant-growth-promoting rhizobacteria of the genus *Azospirillum* have been used as a model object for study of plant-microbial associativeness owing to their abilities to fix atmospheric nitrogen, to synthesize phytohormones, and to influence plant water status and also owing to other positive factors. In studying associative symbioses, it is important to reveal the associated partners’ active components that characterize the effectiveness of this interaction. The outer-membrane lipopolysaccharide (LPS) of gram-negative bacteria of the genus *Azospirillum* has an important role in the formation of associative bacterium-cereal root interaction. In particular, *Azospirillum* LPS induces specific deformation of the wheat-seedling root-hairs, as happens in the presence of whole bacterial cells (Fedonenko et al. 2001). In addition, *Azospirillum* LPS causes an increase in the synthesis of major proteins in the wheat-root cell apoplast, comparable with the action of intact bacterial cells (Matora et al. 1995).

Currently, few data exist on the functional activity of plant-root apical meristems during plant interaction with the associated micropartners, although it is these organs that serve as formative and regulatory centers in the host plant and are a major site of localization of associative bacteria. This work examined the functional activity of wheat-seedling-root meristems during treatment with the LPS isolated from the outer membrane of *A. brasilense* strain Sp245, as compared with inoculation with whole bacterial cells.

Etiolated 3-day-old wheat seedlings were incubated for 24 h either in a solution of 10 mg/l LPS or in a bacterial suspension (cell density, $10^9$ cells/ml). The control was noninoculated plants grown in water culture. Samples were taken at 2 days after inoculation. The functional activity of the seedling-root-tip meristem cells was assessed by using two parameters: (1) the results of determination of the cells’ mitotic index and (2) comparative estimation of the content of the proliferative antigen of initials (PAI) – a molecular marker of wheat-meristem cells (Evseeva et al. 2007). For determination of the mitotic index of the root-apex meristematic cells, the material was fixed in acetic-acid-ethanol (1:3), stained with acetohehantoxylin, macerated with the cytase enzyme, and visualized at 400X magnification. PAI was revealed with an immunochemical test-system developed by us on the basis of the enzyme immunoassay using rabbit monospecific anti-PAI antibodies.

Inoculation of the wheat-seedling roots with whole bacterial cells led to a 2-fold increase in the root-cell mitotic index and to an approximately 1.5-fold increase in PAI content in the cells, as compared with the noninoculated plants. When the wheat-seedling-root system was treated with the isolated LPS, the mitotic index of the root-meristem cells was increased 2.4-fold and PAI content was increased 1.4-fold.

In summary, the increase in PAI content recorded after root inoculation with whole bacteria and also after root treatment with the isolated bacterial LPS is associated with the fact that the cell divisions in the root meristems of inoculated plants proceed more intensively. This possibly facilitates the formation of new adventitious roots and leads to the well-known growth-promoting effects exerted by associative bacteria. Possibly, LPS may be considered to be an active component of the *Azospirillum* cell surface that determines contact bacterium-wheat root interactions and also is involved in the processes inducing plant responses to these interactions.

References.


A n n u a l  W h e a t  N e w s l e t t e r  V o l .  5 5 .


ITEMS FROM SPAIN

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*Localization of two class-III peroxidase genes expressed in the roots of a Heterodera avenae-resistant wheat line.*

The cereal cyst nematode is a pest that seriously affects cereal crops in many of the world’s wheat-growing areas. The *H. avenae* resistance gene *Cre2* from *Ae. ventricosa* present in the *Ae. ventricosa*/*wheat* introgression line *H-93-8*, was shown to confer a high level of resistance to the Spanish pathotype Ha71 (Delibes et al. 1993). The infection of *H-93-8* line with *H. avenae* resulted in a hypersensitive reaction, with syncytial cells deteriorating in a few days. Following nematode infection, peroxidase, esterase, and superoxide dismutase activities increased in *H-93-8* roots compared with the parental, susceptible cultivar Almatense, *H-10-15* (Andrés et al. 2001). Twenty peroxidase genes were characterized from 211 ESTs and 259 genomic DNA clones of this resistant line. The alignment of deduced amino-acid sequences and phylogenetic clustering with peroxidases from other plant species showed that these enzymes fall into seven different groups (designated TaPrx108 to TaPrx114) that represent peroxidases secreted into the apoplast by a putative N-terminal peptide signal. The expression levels of groups *TaPrx112* and *TaPrx113* in roots of the *H-93-8* resistant line increase in response to nematode infection. The maximum peroxidase levels were reached four days post-inoculation. Moreover, the expression of groups *TaPrx112* and *TaPrx113* always was much higher in *H-93-8* line (4- and 100-fold, respectively) than in their susceptible parental. This fact may be related to a constitutive mechanism of defense in this resistant line. The chromosomal assignment of peroxidases of both groups was done using Sears’ aneuploid wheat lines (Sears 1954; Kimber and Sears 1968) and PCR-specific primers from peroxidases. Two PCR fragments obtained from peroxidases *TaPrx112-F* and *TaPrx113-F* were absents in nulli-tetrasomic and ditelosomic lines N2BT2D and Dt2BL, respectively. Therefore, both peroxidase genes would be located in 2B short arm chromosome of wheat.