Yellowish - brown changes on leaves of winter rye
(Secale cereale L.)

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SUMMARY - Two mutants of winter rye (Secale cereale L.) (hs1 and hs2) with yellowish-brown
changes on leaves are described. Both forms were characterized by a lower number of tillers, but the
height of plants and length of ears were similar to those in control plants. Mesophyll cells of these
mutants the number of chloroplasts and grana and the chlorophyll content were lower than those in
dark green plants. Genetic analysis of F1, F2 and backcross generations have proved that the trait of
yellowish broW11 changes is determined by the same recessive gene. This gene is designated ns after
term “yellow necrotic spots”.

INTRODUCTION

Chlorophyll mutations were observed in winter rye after self-pollination and mutagenesis
(MUNTZING 1963; KUBICKI and KUBICKA 1981; KUBICKA 1986, 1994). Some of these
changes (lethal) appeared mostly in young seedlings. Leaves of plants with these chlorophyll
changes were white, yellow or pink in colour (SMIRNOV and SOSNICHINA 1984; KUBICKA
Other mutants were characterized by partial changes of the leaves during the whole development
of plants to maturity (SZILKO and KEDROV-ZIHMAN 1982; DE VRIES and SYBENGA 1984;
GABARA and KUBICKA 1991). ZEVEN (1981) described a necrosis trait in wheat which
occurred only in hybrid plants. These phenotypes died before the heading state. The hybrid
necrosis in wheat was determined by two complementary, dominant genes. Later the analogous
genes were discovered in rye and triticale by REN and LELLEY ( 1990) . In contrast, most of
chlorophyll changes in rye were inherited by monogenic, recessive genes (BREWBAKER 1926;
DE VRIÊS and SYBENGA 1984; SMIRNOV and SOSNICHINA 1984; KUBICKA et al. 1986;
In plants with reductions in leaf chlorophyll, chloroplasts were either absent (in albino forms) or reduced in number within individual cells was observed (GABARA and KUBICKA 1991). For example, the feature of light green colour of winter rye leaves was caused by lower number of chloroplasts (GABARA and KUBICKA 1991) but chlorophyll changes in leaves of *Hordeum* were the consequence of diminution in chlorophyll content (WATANABE et al. 1995). In the present paper we describe the morphology of winter rye with light yellow necrotic pattern on leaves and the inheritance of this feature. Moreover, we tried to find out whether the above trait resulted from the changes in number and ultrastructure of chloroplasts and/or chlorophyll content.

**MATERIAL AND METHODS**

Two lines with leaves characterized by irregular yellowish-brown changes were the object of the present studies. The first line (hs1) was selected in generation S2 after self-pollination of material originating from SHR Jeleniec. Then after numerous self-pollinations the homozygotic plants obtained in generation SIO were crossed with control ones (with uniformly green leaves) originating from inbred line of SIO generation. The second form, mutant (hs2) morphologically similar to hs1 plants, was obtained after treatment of inbred line (self-fertile, of generation S - L 299) with 1.5 mM azide sodium. Mutant hs2 was crossed with control plants and the test for allelism was performed (crosses between forms hs1 x hs2). Plants of generations F1, F2 and backcrosses were observed and reliability of the obtained results was checked by means of chi-square test.

The following features: height of plants, length of ear and tillering were analysed in the twenty control plants as well as in twenty hs1 and hs2 forms, each. The results were verified with "t" Student test.

Chlorophyll content was determined by extraction with 80% acetone according to BRUINSMA (1963) and measured at 645, 652 and 663 nm in spectrophotometer Spectronic 601 (Milton Roy Company USA).

The number and ultrastructure of chloroplasts were studied in mesophyll cells from the same part of control leaves and of hs1 and hs2 plants. The number of chloroplasts was counted in material fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer and macerated in 0.05 M EDTA in 0.35 M sucrose, pH 9.0, at 60° C for 4 h (POSSINGHAM and SMITH 1972). Chloroplast ultrastructure was analysed in the material fixed and dehydrated in standard vap. as described previously (KUBICKA et al. 1986). The specimens were embedded in Epon 812, sectioned with a glass knife on an LKBultramicrotome, stained in uranyl acetate followed by lead citrate and examined in a Jeol electron microscope at 80 kV.
RESULTS

Morphology of plants

Plants of both forms, hs1 and hs2, had similar irregular white-yellow necrotic patterns on the leaves. This feature appeared in the early spring (the end of March) and was visible until the leaves started to dry. In comparison to the control material the forms hs1 and hs2 were characterized by low tillering, but there was no difference in height of plants or in length of ears (Table 1). Since both forms (hs1, hs2) produced grains after self- and open-pollination there were no difficulties with their maintaining homozygotes.

TABLE 1. - Biometric measurements of selected features in control plants (0, hs1 and hs2 forms.

<table>
<thead>
<tr>
<th>Feature</th>
<th>control plants</th>
<th>hs1</th>
<th>NUR* at α= 0,05</th>
<th>hs2</th>
<th>NUR* at α= 0,05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height of plants (in cm)</td>
<td>90.7</td>
<td>89.3</td>
<td>2.3</td>
<td>92.5</td>
<td>2.4</td>
</tr>
<tr>
<td>Tillering (in pieces)</td>
<td>13.8</td>
<td>8.0</td>
<td>2.1*</td>
<td>9.3</td>
<td>1.7*</td>
</tr>
<tr>
<td>Length of ear (in cm)</td>
<td>7.5</td>
<td>7.1</td>
<td>0.7</td>
<td>7.6</td>
<td>0.6</td>
</tr>
</tbody>
</table>

* - NUR between control and each form
/+/ - significant difference

Chlorophyll content

The content of both chlorophylls (a + b) in dark green plants underwent a significant reduction in both forms studied, particularly in hs2 (Fig. 1).

Number of chloroplasts and ultrastructure

The number of chloroplasts per mesophyll cell in hs1 form exceeds that in hs2, although in comparison to control material a reduction in chloroplast number was noticed (Fig. 1) in both forms.

Electron microscopy did not reveal significant differences in ultrastructure of chloroplasts in the studied plants. Chloroplasts of all plants were typical in structure with well developed granal and intergranal; thylakoids (Figs. 2-4). However, contrary to control material, in chloroplasts of hs and hs2 the grana were less numerous and the number of thylakoids per single granum was smaller in these plants, particularly in hs (Fig. 5).
Genetic analysis of yellow-necrotic spot feature

After crossing both forms with control plants, the F1 generation had green leaves containing single slightly visible small dots. On the other hand, in the F2 generation segregation into plants with green leaves and those with yellow-necrotic pattern on leaves similar to the theoretical ratio 3:1 was observed. This result suggests that one gene, with recessive inheritance controls the yellow-necrotic trait (Table 2). The above observations were supported also by segregation obtained in backcrosses. Tests for allelism (crossing of both forms together) show 100% of plants similar to parental forms in the generation F1. Thus, results obtained indicated that the occurrence of yellow necrotic spots on leaves in both forms (hs1 and hs2) was determined by the same gene, which was designated with the symbol $yns$ after the term "yellow necrotic " spots.

DISCUSSION

In the present paper we described two forms of winter rye (*Secale cereale* L.) characterized by irregular, yellow-necrotic spots on the leaves. The first of these forms (hs1) was selected in inbred generation while, the second one obtained in M postmutated generation. The trait of yellow necrotic spots on the leaves in these forms appearing in the heading stage was visible until leaves dried. These plants were able to set seeds after self-pollination, there was less tillering than the control plants.
Fig. 2. - Chloroplast from control dark-green leaves. x 30000. - Fig. 3. - Chloroplast from hs, form. x 24400. - Fig. 4. - Chloroplast from he form (mutant). x 26400.
TABLE 2. - Genetic analysis of plants obtained after crossing control plants (C) with hs\textsubscript{1} and hs\textsubscript{2} forms

<table>
<thead>
<tr>
<th>Parents and generations</th>
<th>Obtained</th>
<th>Expected</th>
<th>( \chi^2 )</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>hs\textsubscript{1}</td>
<td>hs\textsubscript{2}</td>
<td>C</td>
</tr>
<tr>
<td>P\textsubscript{1} - K</td>
<td>60</td>
<td>60</td>
<td></td>
<td>60</td>
</tr>
<tr>
<td>P\textsubscript{2} - hs\textsubscript{1}</td>
<td>100</td>
<td>60</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>P\textsubscript{1} - hs\textsubscript{2}</td>
<td>870</td>
<td>275</td>
<td>838.75</td>
<td>286.25</td>
</tr>
<tr>
<td>F\textsubscript{1} (P\textsubscript{1} × P\textsubscript{2})</td>
<td>143</td>
<td>138</td>
<td>140.50</td>
<td>140.50</td>
</tr>
<tr>
<td>F\textsubscript{2}</td>
<td>475</td>
<td>157</td>
<td>471</td>
<td>153</td>
</tr>
<tr>
<td>B\textsubscript{1} (F\textsubscript{1} × P\textsubscript{2})</td>
<td>91</td>
<td>300</td>
<td>85</td>
<td>88</td>
</tr>
</tbody>
</table>

Fig. 5. - Number of grana and thylakoids in chloroplasts from leaves of chlorophyll mutants

According to ZEVEN (1981) feature of hybrid necrosis in wheat was determined by two complementary, dominated genes \textit{Ne1 Ne2}. These genes were located on 5B and 2B chromosomes, respectively. Analogical phenomenon was observed in rye and triticale by REN and LELLEY (1990). The genes responsible for the appearance of hybrid necrosis in rye designated by symbols \textit{Ner1} and \textit{Ner2} were located on the chromosomes 5R and 7R. The plants with the latter genotypes died before the heading phase. Another mode of inherit -
ance of irregular, necrotic spots on the leaves occurring in two forms of winter rye was 
demonstrated in this paper. A genetic analysis performed on the F1 and F2 generations as well as 
backcrosses have shown that this feature was determined by a single, recessive gene. The results 
derived from crosses of both forms (test for allelism) showed that all plants in the F1 generation 
were morphologically similar to the parental forms. This fact indicated that the trait of yellow 
necrotic spots was determined by the same recessive gene although the forms had a different 
origin. Therefore this gene was denoted by a symbol \( \text{yns} \) after the term "yellow necrotic spots". 
Analogically to analysed features most of chlorophyll mutations in rye (BREWBAKER 1926; 
MUNTZING 1963; DE VRIES and SYBENGA 1984; SMIRNOV and SOSNICHINA 1984; 
KUBICKA 1986, 1994; GABARA and KUBICKA 1991; GABARA et al. 1991) were 
determined by a single, recessive gene. WATANABE et al. (1995) found that lower chlorophyll 
content was responsible for light green leaves in \( \text{Hordeum vulgare} \). On the other hand, our earlier 
studies demonstrated the reduction in the number of chloroplasts in light green plants of rye 
(GABARA and KUBICKA 1991). Results of the present paper showed a significant diminution 
in both chlorophylls \( (a + b) \) accompanied by decrease in the number of chloroplasts, especially in 
hs2 form. The gene \( \text{yns} \) appeared responsible for the reduction in the numbers of chloroplasts, 
grana and thylakoids per single granum in both forms but mostly in hs2, as compared to the 
control. This fact can be explained only by the different genetic background of both investigated 
forms. The described types of chlorophyll changes are not of practical importance, but they seem 
to be of interest from the theoretical point of view. Their analysis would enrich the knowledge 
about rye, which is insufficiently genetically studied.

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