MOLECULAR AND PHENOTYPIC CHARACTERIZATION OF ROMANIAN WINTER WHEAT (*TRITICUM AESTIVUM* L.) LINES IN CONTEXT OF PRE-HARVEST SPROUTING

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**Abstract.** The main aims of our study are the genotypic and phenotypic analysis of four Romanian wheat (*Triticum aestivum*) lines. The results will be used in further breeding studies for increasing the pre-harvest sprouting (PHS) resistance in wheat. PHS occurs when the germination of the seed on the spike starts in the field before harvest because of high humidity and weather conditions. This damage causes economic and yield losses all over the world. The four investigated parental lines, with different sensibility to PHS, were Turda 95 (sensible), Turda 18-94 (resistant), Lovrin 32 (sensible), Fundulea 29 (resistant). The molecular study was performed with 640 SSR markers. The results showed that between Turda 95 and Turda 18-94 line 90 primers were polymorphic (14%), between Turda 95 and Fundulea 29 line 75 primers were polymorphic (11.17%) and between Turda 18-94 and Fundulea 29 line 83 primers were polymorphic (12.97%). The result from the phenotypic characterization of heading date and plant height showed that the F2 population of the cross Turda 95 and Turda 18-94 has normal distribution, and the PHS scoring shows bimodal distribution and these data can be used for next analysis.

**Keywords:** microsatellite markers, polymorphism, pre-harvest sprouting, grain quality, *Triticum aestivum* L.

**INTRODUCTION**

Wheat (*Triticum aestivum*) is one of the most important crop plants, the bread made from wheat flour it represents a major food in more than half of the world population. Wheat is used for leavened bread, flat and steamed breads, biscuits, cakes, pasta, noodles, couscous and beer. The raised bread loaf is possible, because the wheat kernel contains gluten, an elastic form of protein that traps minute bubbles of carbon dioxide when fermentation occurs in leavened dough, causing the dough to rise (Hanson et al., 1982). Unfortunately wheat it is sensitive to abiotic stresses and pathogens. An important damage is the germination of the wheat seeds on the spike in the field before harvest at high humidity conditions, which is called pre-harvest sprouting (PHS). Pre-harvest sprouting is a damage caused by environment and gene interaction.

In PHS damaged wheat the alpha-amylase activity is high and this causes the degradation of the starches and the result is a low quality of the bakery products and bread. Therefore PHS causes economical and yield losses in the quality of kernels, thus limiting their end use. Damaged kernels are a popular source of animal feed, particularly in years when harvests are adversely affected by rain and significant quantities of the grain are made unsuitable for food use. Such low-grade grain is often used by industry to make adhesives, paper additives, and several other products and even in the production of alcohol.
Genetically speaking, PHS heredity is treated as quantitative trait. Seed dormancy is conditioned by the pleiotropic effects of gene R, which determines the red pericarp of the seeds but also by genes that control the physiological process of germination and have a marking effect on the embryo dormancy. Therefore, PHS is controlled both by embryo physiology and also by seed coat regulation, each caused by different genetic systems (Gross et al., 2002).

Breeding for resistance to pathogens and damages including pre-harvest resistance is an important and attractive research topic worldwide to increase the quality of wheat (Chen et al., 2007; Groos et al., 2002; Lohwasser et al., 2005; Liu et al., 2008).

A phenotypic characterization of some crosses and backcrosses of Romanian wheat was performed by Lupu et al. (2010, 2011).

The molecular and phenotypic research is needed in the improvement of a good quality winter wheat in Romania and to reduce the time of breeding.

The simplicity and easiness of the SSR (Simple Sequence Repeats or microsatellites) markers made this method useful in plant breeding. SSR markers are often utilized in molecular studies of the wheat (Zeb et al., 2009) especially that there are many SSR markers special designed for wheat (Röder et al., 1998; Pestsova et al., 2000; Somers et al., 2004; Ganal and Röder, 2007)

The first aim of this study was to fulfill the lack of knowledge of the polymorphic percentage in four Romanian wheat lines.

The second aim was to make the phenotypic characterization of the two parental lines, with highest polymorphic percentage, and the F2 population of these if they have normal distribution.

**MATERIAL AND METHODS**

The plant material, four parental lines with different sensibility to PHS (Turda 95-sensible, Turda 18-94-resitant, Lovrin 32-sensible, Fundulea 29-resitant) and three crosses between the parental lines (Turda 95 x Turda 18-94, Turda 95 x Fundulea 29, Turda 18-94 x Lovrin 32) was obtained from ARDS Turda Romania and the research was made in Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, Germany.

Genotypic characterization. The DNA isolation from parental lines and later from the F2 population was performed according to the protocol of Doyle and Doyle (1990) from two week old leaves.

The characterization for polymorphism of the parental lines was conducted with 640 SSR (Simple Sequence Repeats or Microsatellite) markers (gwm, wmc and barc) specific for wheat (Röder et al., 1998; Ganal and Röder, 2007) and a Viviparous-1 allelic variant primer (Vp1-B) (Yang et al., 2007b). For the SSRs the left primer was fluorescence labeled and the label was recognized by the laser from the Automated Laser Fluorescent (ALF) fragment analyzer. Fragment analysis was carried out as described in Röder et al. in 1998. The Vp1-B marker was tested on agarose gels as described by Xia et al. in 2008. Marker which showed polymorphism between the two parental lines was used for studying the F2 population with the highest percent of polymorphism, which was the cross between Turda 95 and Turda 18-94. This population was used for further phenotypic characterization (Vas et. al, 2013)

Phenotypic characterization. The cross between Turda 95 and Turda 18-94 was scored for the heading date and plant height. PHS was checked from freshly harvested
mature ears stage (Rehman *et al.*, 2012) kept in humid conditions in wet sand for 14 days. PHS was scored from 1 to 7 (where 1 was no sprouting and 7 indicated 100% sprouting) (Figure 1).

**RESULTS AND DISCUSSION**

**Results from genotypic analysis.** The molecular study was performed with 640 SSR markers. The polymorphic analysis is important, because we can see in the population the differences between two parental lines. The results from the analysis showed that between Turda 95 and Turda 18-94 parental lines 90 primers were polymorphic (14%), between Turda 95 and Fundulea 29 parental lines 75 primers were polymorphic (11.17%) and between Turda 18-94 and Lovrin 32 parental lines 83 primers were polymorphic (12.97%).

The results of the analysis for polymorphism from parental lines suggest that the highest percentage of polymorphism is between Turda 95 and Turda 18-94 (Table 1, Vas *et al.*)

<table>
<thead>
<tr>
<th>Parental Lines</th>
<th>Polymorphic Percentage</th>
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</thead>
<tbody>
<tr>
<td>Turda 95 x Turda 18-94</td>
<td>14%</td>
</tr>
<tr>
<td>Turda 95 x Fundulea 29</td>
<td>11.17%</td>
</tr>
<tr>
<td>Turda 18-94 x Lovrin 32</td>
<td>12.97%</td>
</tr>
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**Results from phenotypic characterization.** Result from the phenotypic characterization with statistical program SigmaPlot (Figure 2) showed that the distribution is normal (Gaussian distribution) for heading date and plant height.
The data, from scoring the PHS with direct method, illustrate a not normal, bimodal distribution (Figure 3.), which can indicate an action of a major gene.

CONCLUSION

The investigation of Turda 95, Turda 18-94, Fundulea 29 and Lovrin 32 autochtonous wheat lines is a big step for the winter wheat research in Romania.

The polymorphic data describe the genetic diversity between the four parental lines and this can be further used for QTL mapping studies to establish markers for marker-assisted selection.

The cross between the two parental lines Turda 95 and Turda 18-94 is good for further morphological and molecular analysis and a future QTL-analysis.

ACKNOWLEDGMENT

We would like to thank Deutsches Bundestiftung Umwelt (DBU) for funding Eszter Vas stay in Germany at IPK Gatersleben.
REFERENCES


