

# Agronomic Properties Assessed for Population of Recombinant Inbred Lines of Rye (*Secale cereale* L.) with Known Responses to Nutrient Deficiency Stress at the Seedling Stage

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## Abstract

The experiment was established and carried out in light and slightly acidic soil in the years 2008-2010. The research material included one hundred thirty-eight RILs ( $F_{8:10}$ ) of rye with known response to nutrient stress were determined at the seedling stage obtained *in vitro* in mature embryos cultures and in two parental inbred lines and  $F_1$ . The following factors were determined in the full maturity stage of each RIL: plant height, spike length, plant tillering, spikelet number per spike, grain number per spike, spike fertility, grain weight per spike, grain weight per plant and 1000 grain weight. Differences in the examined traits were found between parental inbred lines, and the heterosis effect was observed in  $F_1$  hybrid. The range of variability of the examined functional traits demonstrated in the population of RILs proved the transgression effect. The analyzed RILs were arranged into six groups and described with the use of Ward's agglomerative method and grouping variables: spike fertility, grain weight per spike and per plant, thousand grain weight. Significant differences were found among other things between spike morphology and fertility traits and yield per a plant. The possibility of selection of RILs with extreme traits described both in the field and laboratory experiments in comparison with known tolerance to nutrient stress was described. The obtained correlation coefficients for the examined functional traits of RILs were in accordance with those published in reference literature. The correlation coefficients determined for selected seedling traits from the laboratory experiment and for selected traits of a mature plant may prove the usefulness of the test in studies on selection of genotypes desired for sustainable agriculture or for research on heritability of traits of tolerance to nutrient stress.

**Keywords:** agronomic properties, nutrient deficiencies, RIL, *Secale cereale*, selection

## Introduction

Breeding programs are implemented for economically important cultivated plant species, aiming at obtaining cultivars tolerant to stresses caused by biotic and abiotic factors (Górny *et al.*, 2011; Messmer *et al.*, 2011; Wolfe *et al.*, 2008), including stress of drought (Rapacz *et al.*, 2010; Sayar *et al.*, 2010), cold (Rapacz *et al.*, 2008), salinity (Sayar *et al.*, 2010), toxic influence of aluminum (Anioł and Gustafson, 1984) or nutrient deficiencies (Cieply and Oracka, 2000; Liu *et al.*, 2008; Lynch, 2007; Rzepka-Plevneš *et al.*, 1997a, 1997b; Rzepka-Plevneš and Tomczak, 1998). Since plants are able to utilize only about 30% of available nitrogen doses, high doses of plant mineral fertilization were found to increase only the cost of plant cultivation (Lynch, 2007; Raun and Johnson, 1999; Zhou and Chen, 2002). The remaining doses contribute to ground water contamination (Raun and Johnson, 1999), eutrophication of rivers and lakes, and adversely affect produced food (Lynch, 2007; Zhou and Chen, 2002).

A way to minimize the mentioned threats is introduction of cultivars tolerant to stress to cultivation, including to stress caused by nutrient deficiencies (Górny *et al.*,

2011; Löschenberger *et al.*, 2008; Rzepka-Plevneš *et al.*, 1997b, Wolfe *et al.*, 2008). It was demonstrated that effective utilization of nitrogen ions by a plant depends on availability of potassium ions (Ashraf and Harris, 2005; Tsay *et al.*, 2011). Species of cultivated plants have a wide range of genotypic variability predisposing a plant to growth under the conditions of decreased mineral fertilization, and an important factor that determines this adaptation is a complex and genetically complicated trait - root architecture. Selection of genotypes adapted for growth under conditions of nutrient deficiencies is carried out depending on root system (Löschenberger *et al.*, 2008; Lynch, 2007; Messmer *et al.*, 2011; Tuberosa and Salvi, 2007). Genotypes develop a long and well-developed root system demonstrate high effectiveness of exploration of deeper soil layers (Lynch, 2007), thereby increasing a chance of avoiding drought or obtaining nutrients (Bloom *et al.*, 1985). According to many authors, the assessment of genotype response to nutrient stress carried out under laboratory conditions in the seedling stage is reflected in a similar response in the plant maturity stage (Liu *et al.*, 2008; Messmer *et al.*, 2011), although some authors have different opinions (Bolanós *et al.*, 1993).

Cultivated rye (*Secale cereale* L. ssp. *Cereale*) is a species adapted to growth in poor soils. In the case of its cultivation the problem of over-fertilization practically does not exist (Arseniuk and Oleksiak, 2003; Geiger and Miedaner, 2009; Górny *et al.*, 2011). However, it should be noted that its cultivation is displaced by triticale from good and medium soils into poorer soils (Dmochowska, 2011; Górny *et al.*, 2011; Rzepka-Plevneš *et al.*, 1990). Such a situation is taking place in the countries of the RYE-BELT region. In Poland in 2000 rye accounted for 17.2% of all crops, in 2010 - 10.1%. In the case of triticale the situation is opposite - in 2000 it accounted for 5.6% of total crops, while in 2010 - already 12.5% (Dmochowska, 2011).

Rzepka-Plevneš *et al.* (1997a, 1997b) showed great variability within open-pollinated populations, strains or inbred lines in terms of their response to nutrient stress caused by nitrogen and potassium deficiencies in *in vitro* cultures of mature embryos as well as in hydroponics (Rzepka-Plevneš and Tomczak, 1998), while their assessment of the functional value of selected populations (genotypes) both in the pot (Rzepka-Plevneš and Tomczak, 1998) and field experiment showed usefulness of the methods in selection (Rzepka-Plevneš and Kulpa, 1996). Rzepka-Plevneš *et al.* (1997b) stated that the populations selected under low N supply in soil were characterized by high spike productivity, TGW and grain field per pot. Similar results for corn were presented by Liu *et al.* (2002) and Tuberosa *et al.* (2002), who demonstrated significant correlations between root system morphology and NUE (Nutrient-Use Efficiency), and at the same time identified a range of QTLs linked to development of that trait (Liu *et al.*, 2008; Malamy 2005; Tuberosa and Salvi, 2007).

Possessing genotypes tolerant to stress caused by nutrient deficiencies is a significant element of the breeding processes aiming at obtaining new cultivars of cultivated plants, including cereals, adapted to cultivation in a sustainable agriculture system (Górny *et al.*, 2011; Löschenberger *et al.*, 2008; Rzepka-Plevneš *et al.*, 1997b; Wolf *et al.*, 2008). Such genotypes are an interesting material for research on mechanisms of heritability of the trait. Segregating descendant populations ( $F_2$ ) and also combinations of highly homogeneous RILs obtained as a result of crossing genotypes extremely different in the examined trait facilitate identification of molecular markers linked to QTL of a given trait or identification of major genes determining it (Lammerts van Bueren *et al.*, 2010; Masojć *et al.*, 2009).

The aim of the present study was to describe selected agronomic traits of the population of 138 recombinant inbred lines of rye in the field experiment and to make an attempt at describing correlation relationships between morphology of a mature plant and response of individual RILs to nutrient stress in the seedling stage, determined in some other experiment.

## Material and methods

The research was carried out in the years 2008-2010 in the vegetation hall of the Agricultural Academy in Szczecin, at present named the West Pomeranian University of Technology in Szczecin (Poland).

### Plant material

Research material included a population of 138 recombinant inbred lines (RILs) of rye derived from the  $F_{2,3}$  generation obtained as a result of crossing inbred lines 153/79-1 and Ot1-3 (each,  $S > 23$ ). Characterization and origin of parental lines were presented by Rzepka-Plevneš *et al.* (1997a). RILs of rye were derived from the  $F_3$  generation with the use of the SSD method (*Single Seed Descent*). Characterization of biometric traits of RILs was started after six years of inbreeding and addressed the subsequently obtained  $F_{8,10}$  generations. Also parental lines and annually obtained  $F_1$  hybrids were under observation.

### Field conditions

The experiment was established in light soil with slightly acidic reaction (pH KCl  $\approx 5.8$ ) in a stand after many-year rye cultivation. Fertilization with nitrogen was applied pre-sowing during plowing in the dose of respectively 30 kg N ha<sup>-1</sup> and as top-dressing in the tillering stage in the dose of 15 kg N ha<sup>-1</sup>. Fertilization with phosphorus and potassium was not used because soil analyses showed P in the amount of 24, K - 7.6, Mg - 4.8, Mn - 15; Cu - 0.89; Zn - 16 and Fe in the amount of 120 mg per 100 g of soil collected for the analysis.

### Experiment design

Biometric traits of RILs were assessed in a microplot experiment established in the system of complete randomization in three replications, i.e. two-row plots with the length of 90 cm and spacing of 18 cm. The plants were manually planted in the 1-2 leaf stage, depending on weather conditions, in the last week of September or in the first week of October. Ten plants were growing in one row. The plants were protected against diseases and pests with the use of spraying with the preparations: Punch Bis 400 EC, Calypso 480 SC, Mospilan 20 SP and Mesurool Schneckenkorn 04GB according to the doses recommended by the producers. Weeds were removed manually and no growth regulators were applied. In the earing stage, isolators were put on spikes of the longest stalks of six plants uniform in height of each RIL. Also isolators were put on all spikes of three out of six representative plants of a given RIL. Grain of one of them was intended for establishing the experiment in the following year. In the case of an insufficient amount of grain required for establishing the experiment, a lower number of plants was sown in replications.

*Measurement of plant traits*

For these plants the following traits were assessed in the full maturity stage: plant high (PH) in cm; spike length (SL) in cm; plant tillering (PT); spikelet number per spike (SNS); grain number per spike (GNS); spike fertility (SF) in %; grain weight per spike (GWS) in g; grain weight per plant (GWP) in g, and 1000 grain weight (TGW) in g. Statistical analysis. The assessment of variability of the examined traits in the following years was carried out on the basis of descriptive statistics. Arithmetic mean ( $\bar{x}$ ), standard deviation (SD), range (D), bias and kurtosis were calculated for parental lines,  $F_1$  and the population of RILs. Two-ways analysis of variance in a completely randomized design was carried out separately for the traits of parental lines,  $F_1$  (G), years (Y) and interactions (G  $\times$  Y), separately for RILs, and the results were presented in the form of syntheses. The differences between means were examined with the use of *t*-Tukey's test with  $p < 0.05$  and  $p < 0.01$ . Concordance with normal distribution for individual traits of the population of RILs was illustrated on the basis of analysis of stemplots and histograms with marked normal distribution curve. Significance of deviation of trait distribution from normal distribution was analyzed with the Kolmogorov-Smirnov test. Mean values of the examined traits of RILs for the years were used to calculate simple correlation coefficients. These means were used also in cluster analysis carried out using Ward's agglomerative method with four grouping variables: SF, GWS, GWP and TGW. Cluster analysis enabled division of RILs into groups with similar values, presented as a dendrogram on

Tab. 1. Characteristics of agronomic traits of inbred lines of rye and their  $F_1$  hybrid

Genotype Trait	153/79-1	153/79-1 $\times$ Ot1-3	Ot1-3	LSD <sub>0.01</sub>
	$\bar{X} \pm s$			
PH (cm)	124 $\pm$ 2.8 b	137 $\pm$ 4.7 a	95 $\pm$ 2.1 c	1.69
SL (cm)	9.3 $\pm$ 0.5 a	9.6 $\pm$ 0.6 a	6.2 $\pm$ 0.4 b	0.42
PT	5.2 $\pm$ 0.7 b	6.4 $\pm$ 1.2 a	3.6 $\pm$ 0.6 c	0.68
SNS	33.3 $\pm$ 3.1 a	34.4 $\pm$ 3.7 a	26.8 $\pm$ 2.1 b	2.16
GNS	26.1 $\pm$ 3.5 b	47.7 $\pm$ 8.0 a	21.1 $\pm$ 3.4 c	4.69
SF (%)	39.7 $\pm$ 5.9 b	72.7 $\pm$ 14.2 a	38.1 $\pm$ 6.9 b	6.01
GWS (g)	0.7 $\pm$ 0.1 b	1.5 $\pm$ 0.4 a	0.5 $\pm$ 0.1 b	0.22
GWP (g)	3.5 $\pm$ 0.8 b	9.6 $\pm$ 3.8 a	1.8 $\pm$ 0.4 c	1.53
TGW (g)	25.8 $\pm$ 2.8 b	32.2 $\pm$ 10.1 a	24.3 $\pm$ 7.2 b	5.12

Tab. 2. Characteristics of agronomic traits of population of RILs rye investigated in field condition in 2008-2010

2008-2010	Traits	Mean $\pm$ SD	Min	Max	D	CV (%)	Bias	Kurtosis
		PH (cm)	101.3 $\pm$ 11.9	73.8	134.5	60.7	6.2	0.2
	SL (cm)	7.0 $\pm$ 0.6	5.2	9.5	3.9	9.5	0.5	0.7
	PT	3.6 $\pm$ 0.3	2.8	4.1	1.3	7.5	0.2	0.1
	SNS	28.1 $\pm$ 2.1	22.0	34.1	12.1	7.4	0.1	0.1
	GNS	23.9 $\pm$ 3.8	13.5	33.4	19.8	15.9	0.1	0.1
	SF (%)	41.7 $\pm$ 6.9	23.1	62.7	39.5	16.7	0.1	0.4
	GWS (g)	0.5 $\pm$ 0.1	0.3	0.7	0.4	19.7	0.4	0.0
	GWP (g)	1.6 $\pm$ 0.3	0.9	2.4	1.5	20.9	0.2	-0.3
	TGW (g)	18.6 $\pm$ 1.6	15.0	23.4	8.5	8.8	0.3	0.4

the basis of scree graph, assuming the Euclidean distance criterion. The values of the examined traits within the obtained groups of RILs are presented as means and standard deviations, and the differences between the means were analyzed using *t*-Tukey's test on the basis of calculated LSD (Least Significant Difference) with  $p < 0.05$  and  $p < 0.01$ . Statistica 9 PL software was used for the statistical calculations.

**Results**

The genotype of rye inbred lines used as parental components in a combination of crossings of  $F_1$  hybrid (153/79-1  $\times$  Ot1-3) had a highly significant influence on most of their agronomic traits (Tab. 1). Inbred line 153/79-1 had higher values of compared traits, and TGW and SF were exceptions, for which no significant differences were found between the examined lines (Tab. 1). Comparison of traits of the examined inbred lines of rye of  $F_1$  proved that  $F_1$  hybrid outdid the both parental inbred lines in PH, PT, GNS, SF, GWS, GWP and TGW (Table 1). In terms of SL and SNS it did not significantly differ from line 153/79-1 (Tab. 1).

The values of descriptive statistics performed for the examined agronomic traits of RILs are presented in Tab. 2.

In terms of each trait, a range of variability exceeding variability described for parental lines was demonstrated in RILs (Tab. 2). In the individual years of research the values of CVs were different, on average the lowest values

for the population of RILs for the years 2008-2010 were found for PH (6.2%), SNS (7.4%) and PT (7.5%), and the highest for GWP (20.9%, GWS (19.7%) and SF (16.7%) (Tab. 2). It was also demonstrated that distribution of the examined functional traits of RILs did not significantly differ from normal distribution, and the parameters describing distribution functions of individual traits are presented in detail in Tab. 2.

The conducted analysis of variance showed relatively high values of the significant influence of genotypes of the examined RILs of rye and years of research on the values of agronomic traits of the population of RILs in comparison with the values of highly significant genotype-environment interaction (Tab. 3).

The analysis of topology of Ward's dendrogram drawn on the basis of four grouping variables (SF, GWS, GWP and TGW) revealed possibility of division of the population of 138 RILs into six groups ('a'-'f') (Fig. 1).

The number of RILs in individual groups, characterization in terms of each trait, and also results of statistical

analysis for differences between means are presented in Tab. 4.

Significant differences were found between the established groups for six traits: SL, SNS, GNS, SF, GWS and GWP, while for the other three traits (PH, PT and TGW) significant differences were not found (Tab. 4). It was demonstrated that on average the highest values of the examined traits (SL, GNS, SF, GWS and GWP) were attributed to the lines from the 'extreme' group - 'f', and the lowest - to the lines from the 'extreme' group - 'a' (Fig. 1, Tab. 4). No significant differences were observed for SNS trait between groups 'a' and 'f' (Tab. 4).

Fig. 2 depicts characterization of agronomic traits of groups of the population of the RILs examined in the study, established with the use of Ward's agglomerative method in some other experiment (Smolik, 2013). A dendrogram, in which seven groups were distinguished ('a'-'f'), was created with the use of agglomeration of RILs carried out on the basis of means of traits (used as grouping variables) describing morphological response of seedlings

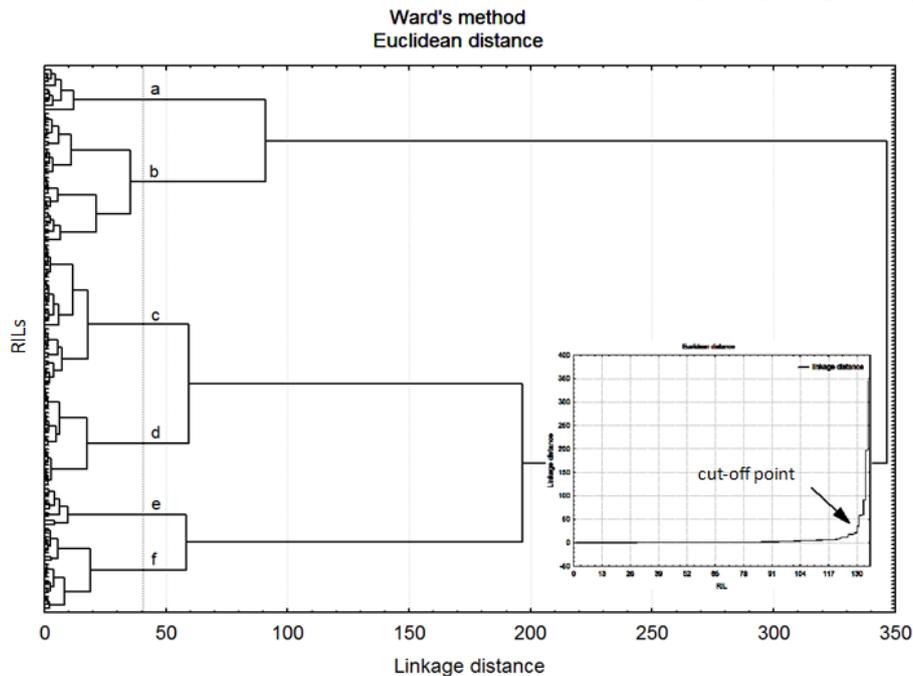


Fig. 1. Ward's dendrogram grouping ('a' - 'f') the population of RILs depending on means of agronomic traits on the basis of four variables: SF, GWS, GWP and TGW into groups 'a' - 'f'. Means of agronomic traits of RILs for the years of research 2008-2010 were used for cluster analysis. The vertical lines indicate the cuts-off used to form the groups

Tab. 3. Analysis of variance for selected agronomic traits of the population of RILs examined in the field experiment in the years 2008-2010

Source	Df	Trait								
		PH (cm)	SL (cm)	PT	SNS	GNS	SF (%)	GWS (g)	GWP (g)	TGW (g)
Y	2	171740**	98**	555**	24789**	58375**	39889**	45**	1205**	6903**
G	135	2107	23.5**	3.41**	242**	775**	2608**	0.43**	5.79**	142**
Y × G	270	505**	5.4**	3.15**	64**	310**	922**	0.19**	3.07**	68**
E	6934	37	0.8	0.56	14	61	190	0.03	0.57	20

Values are the mean squares from ANOVA. \*\* - high significantly different at  $p < 0.01$

Tab. 4. Means of agronomic traits of RILs depending on their affiliation to groups 'a-f' formed on the basis of cluster analysis presented as a dendrogram in Fig. 1

Group	RIL number in group	PH (cm)	SL (cm)	PT	SNS	GNS	SF (%)	GWS (g)	GWP (g)	TGW (g)
a	11	100.2±5.4 a	6.4±0.4 c	3.3±0.2 a	27.2±1.6 b	20.5±2.1 e	34.8±1.7 e	0.4±0.0 c	1.4±0.2 c	18.8±1.3 a
b	33	101.4±6.4 a	6.8±0.7 bc	3.3±0.2 a	28.3±2.5 ab	17.3±1.8 f	28.0±2.3 f	0.3±0.0 d	1.1±0.2 d	18.8±2.2 a
c	36	100.0±5.3 a	7.0±0.6 abc	3.4±0.2 a	28.7±1.8 ab	24.8±1.5 c	43.0±0.6 c	0.5±0.0 b	1.7±0.3 ab	18.4±1.4 a
d	25	102.7±6.9 a	7.0±0.6 abc	3.4±0.3 a	28.6±1.9 ab	22.7±1.6 d	39.5±1.2 d	0.4±0.1 c	1.5±0.2 bc	19.1±1.5 a
e	10	100.3±5.5 a	7.5±0.7 a	3.4±0.3 a	29.9±1.4 a	26.7±2.6 b	47.1±1.9 b	0.5±0.1 ab	1.7±0.4 a	18.0±1.2 a
f	21	101.7±7.6 a	7.4±0.9 a	3.3±0.2 a	29.1±2.4 ab	30.1±2.3 a	55.6±2.7 a	0.6±0.1 a	1.9±0.2 a	18.5±1.4 a
	136	LSD <sub>0.05</sub> =6.09	LSD <sub>0.05</sub> =0.61	LSD <sub>0.05</sub> =0.24	LSD <sub>0.05</sub> =2.00	LSD <sub>0.05</sub> =1.97	LSD <sub>0.05</sub> =1.61	LSD <sub>0.05</sub> =0.06	LSD <sub>0.05</sub> =0.26	LSD <sub>0.05</sub> =1.58

of the same lines to nutrient stress (Smolik, 2013). Group A was presented as mean for 41 RILs of groups 'a'-b, C as mean for 54 RILs of groups 'c'-e, and E as mean for 41 RILs of groups 'f'-g' (Fig. 2).

After "marking" means of agronomic traits of individual RILs, described in the field experiment of the years 2008-2010, on the specific and at the same time unique division of the population of RILs, performed using Ward's agglomerative method after the ending of the laboratory experiment (Smolik, 2013), no significant differences were found between means of most examined traits between the following groups of RILs: A, C and E (Fig. 2).

Higher values of traits were generally found for the lines of group E in comparison with groups A and C, and the statistically significant trait was GWP (Fig. 2). In Fig. 2, letters B and D denote means of the examined traits respectively for 14 lines in each group. They were selected among the RILs determined as susceptible (A) and tolerant (E) in the laboratory experiment (Smolik, 2013). The reason for presenting their characterization in comparison with means of A, C and E (Fig. 2), was the fact that they were also identified in groups of extreme lines (respectively 'a' and 'f') of Ward's dendrogram presented in Fig. 1. It was demonstrated that the groups of selected RILs were sig-

nificantly different in terms of SL, GNS, SF, GWS, GWP (Fig. 2).

On the other hand, no significant differences were found for PH, PT, SNS and TGW, although the group of lines D was better in terms of TGW (Fig. 2).

The analysis of matrix of simple correlations determined between means of traits of the examined population of RILs showed great variability depending on both the values of the correlation coefficients ( $r$ ), and their significance (Tab. 5). They were presented in detail in Tab. 5, indicating high values of correlation coefficients between GWS and GNS (0.90\*\*), SF and GNS (0.89\*\*) and low SL and PH (0.21\*) or TGW and SL (0.18\*) (Tab. 5).

The values of simple correlation coefficients ( $r$ ) between means of traits of seedlings of individual RILs analyzed under laboratory conditions were presented in some other paper (Smolik, 2013).

In the present study the means were used only for generation of matrix of simple correlations between traits of a mature plant ( $F_{8-10}$ ) and traits of the seedlings examined in terms of response to nutrient stress under laboratory conditions ( $F_7$ ). The values of correlation coefficients were low (data not shown). Positive significant correlations were found between PH and coleoptyle length of a seedling growing in a medium with high N and K content ( $r$

Tab. 5. Correlation matrix for agronomic traits of the RIL population

Trait	PH	SL	PT	SNS	GNS	SF	GWS	GWP	TGW
PH	1.00								
SL	0.21*	1.00							
PT	0.00	0.26**	1.00						
SNS	0.26**	0.82**	0.20*	1.00					
GNS	0.13	0.05	-0.07	0.21*	1.00				
SF	0.01	0.32**	-0.15	-0.24**	0.89**	1.00			
GWS	0.27**	0.16	0.02	0.29**	0.90**	0.76**	1.00		
GWP	0.24**	0.24**	0.34**	0.31**	0.79**	0.63**	0.82**	1.00	
TGW	0.37**	0.18*	0.14	0.17	0.25**	0.16	0.63**	0.65**	1.00

Correlation coefficients were calculated for means of agronomic traits of RILs of the years 2008-2010, obtained as a result of analysis of variance for the years 2008-2010. \* -  $p < 0.05$ , \*\* -  $p < 0.01$  significant different from zero

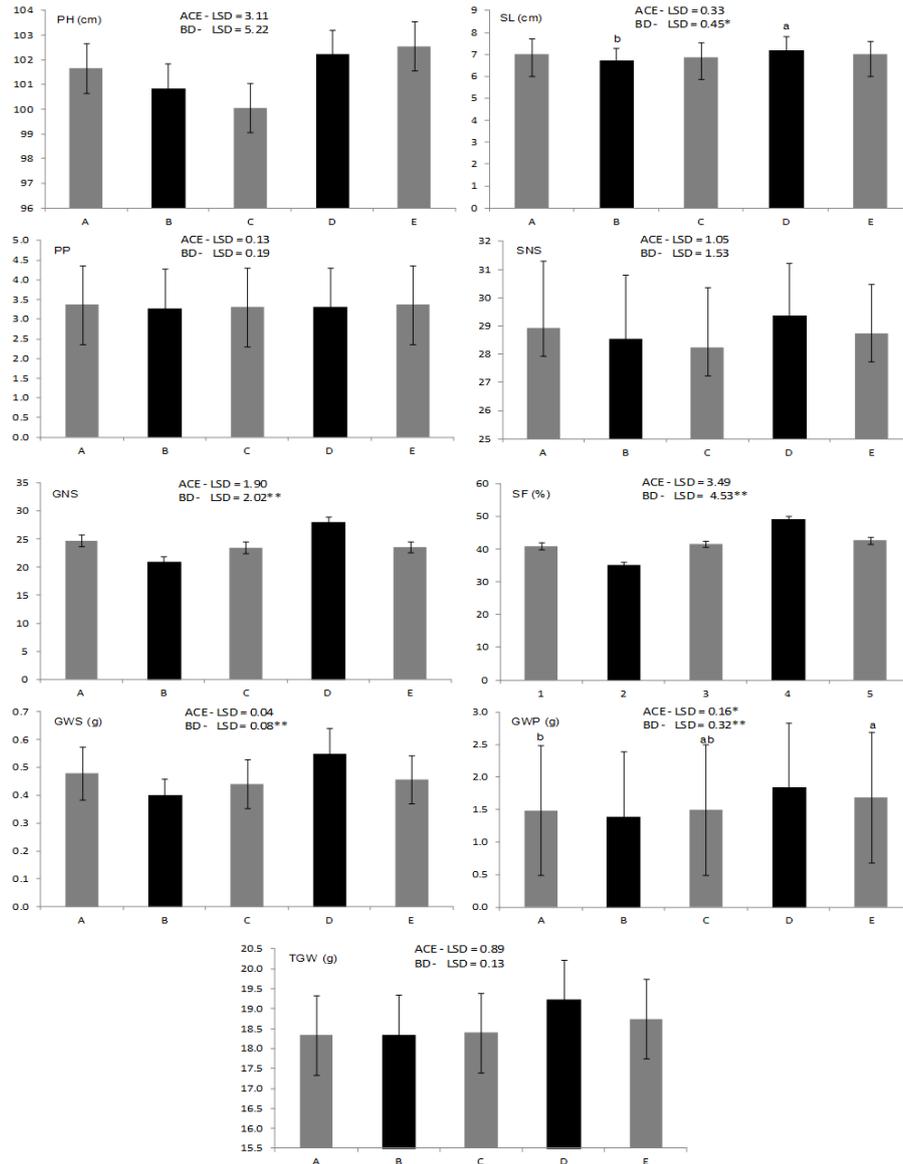


Fig. 2. Means of agronomic traits of the RILs examined in the field experiment in the years 2008-2010. For the groups of RILs denoted by letters A, C, E and B, D, description was placed in the text

PH – plant high; SL - spike length; PP – plant propagation; SNS – spikelet number per spike; GNS – grain number per spike, SF – spike fertility; GWS – grain weight per spike; GWP – grain weight per plant; TGW - 1000 grain weight. The charts show means and standard deviations (SD) of traits of the examined RILs. LSD values were calculated with the use of t-Tukey's HSD test at \*, \*\*  $p < 0.05$ ,  $p < 0.01$ , respectively. Letters 'a' and 'b' denote homogeneous groups

= 0.18\*) as well as between PH and coleoptyle length of seedlings growing in a medium with nutrient deficiencies ( $r = 0.19^*$ ). In addition, positive, yet significant at  $p < 0.10$ , correlations were noted between SNS ( $r = 0.14$ ) and the longest root length (LRL) of seedlings, and also significant correlations were observed between SF and LRL of seedlings growing under conditions of nutrient deficiencies ( $r = 0.19^*$ ). Positive significant correlations were found be-

tween LRL and GWS ( $r = 0.21^*$ ), LRL and GWP ( $r = 0.18^*$ ) and between LRL and TGW ( $r = 0.13$ ) at  $p < 0.10$ . Also a significant correlation ( $r = 0.14$ ) was observed at  $p < 0.10$  between LRL trait of seedlings growing in a high level of N and K in a medium and TGW. Some positive, yet statistically non significant correlations were demonstrated between root number (RN) and PT.

## Discussion

Results of the field experiment, presented in this study, fall into the trend of research on revealing the genetic background of tolerance of cereals, including rye, to abiotic stresses (Masojć *et al.*, 2009), including nutrient stress caused by nitrogen and potassium deficiencies assessed at the seedling stage obtained *in vitro* in mature embryos cultures (Rzepka-Plevneš and Kulpa, 1996; Rzepka-Plevneš *et al.*, 1997a; 1997b), hydroponics and under field conditions (Rzepka-Plevneš and Tomczak, 1998).

The selection of parental components (Ot1-3 and 153/79-1) used for generation of F<sub>2</sub> hybrid, from which the population of 138 RILs (F<sub>8:10</sub>) was derived, was conditioned by extremely different responses of lines to stress caused by nitrogen and potassium deficiencies in a medium (Rzepka-Plevneš *et al.*, 1997a). The lines, as it was shown in the present research, differed significantly also in other agronomic traits, such as PH, SL, SNS, GNS or GWP. Line 153/79-1 is of different origin, shows different response to nutrient stress (Rzepka-Plevneš *et al.*, 1997a) and has different profile of polypeptides - i.e. secalins, separated under the conditions of SDS-PAGE, when compared to line Ot1-3 (Smolik and Rzepka-Plevneš, 2003). Line Ot1-3 has been considered as a line non-tolerant to nitrogen and potassium deficiencies in a medium by Rzepka-Plevneš *et al.* (1997a). It was derived, as one of many inbred lines, by inbreeding of Otello, a Swedish cultivar requiring good stands, selected from one spike of an old population cultivar of rye - Kungs II with its known sensitivity to drought (Persson, 1976). The similar mechanisms of plant response to stress of drought and nutrient deficiencies (Ashraf and Harris, 2005) may confirm also research results by Rzepka-Plevneš *et al.* (1997a), describing inbred line Ot1-3 as susceptible to nutrient stress, and may only suggest the possibility of inheriting the genes that determine lack of tolerance to drought from its descendant cultivar Kungs II (Persson, 1976). On the other hand, inbred line Ot1-3 has low activity of  $\alpha$ -amylase and has been used as a maternal form - donor of resistance genes in research on sprouting in rye (Masojć *et al.*, 2009; Milczarski *et al.*, 2011).

In 2008 the highest mean PH (on average 110.8 cm) for the examined population of RILs was noted in 2008, while in the subsequent years the means were lower. Apart from the potential influence of inbreeding *per se* on the examined trait (Wolski, 1970; Wricke, 1970), also influence of changing weather conditions, such as were noted in the years 2008-2010, cannot be excluded (Miziak *et al.*, 2011). On the other hand, considerable shortening of plant height of rye may be achieved by inbreeding (Wolski, 1970; Wricke, 1970; Zamorska, 1968). According to the authors, inbreeding results in plant height shortening and at the same time has a various influence on spike length. Similar opinion has been shared by Patyna and Grochowski (1972), who reckon that inbreeding enables to break

the correlation between the plant height and spike length (Górny *et al.*, 1982; Łapiński, 1984).

The range of variability for SL ranged between 4.6 and 10.1 cm in the examined population of RILs, and on average for the years it amounted to 7 cm. Similar results of observations were presented by Milczarski (2010). The author reported the range of variability for the three years of research (2004-2006) for PH at the level of 74-146 cm (on average 115 cm) for the mapping population (K) including 73 RILs (F<sub>6</sub>) of rye, where the maternal component was line Ot1-3. In terms of SL the population K had the range of variability at the level from 5.5 to 13.2 cm, amounting to 7.9 cm on average for the years (Milczarski, 2010).

Low variability was demonstrated for PT. The trait ranged from 2.1 to 5.5; on average for the years - 3.6 plants. Similar results for mean for the population K were presented by Milczarski (2010), describing the range of variability from 1.3 to 8.5 plants.

According to Kaczmarek and Pielas (1970) (from Słaboński *et al.*, 1984) and Wricke (1970), SF of inbreeding rye genotypes ranges from 3 to 54% and depends on environmental conditions. Fertility of spikes *per se* at the level of 62 - 82% proves poor grain setting. In the present study SF in the examined population of RILs amounted to 13.8 - 67.6 %, on average 41.7%. In a study by Milczarski (2010) the trait ranged between 2.2 and 82%, reaching the mean of 41.3%. In a study by Słaboński *et al.* (1984), spike fertility for inbred rye lines (S<sub>6</sub>) was from 3.2 to 55.7%. Such large differences in SF may be explained by the effect of genes determining a self-incompatibility type of and lethal and sub-lethal loci in the homozygous state (Kaczmarek and Pielas, 1970; Wolski, 1970; Wricke, 1970).

Spike fertility is reflected in grain number per spike (GNS), and in a further stage in yield per spike (GWS), per plant (GWP) or TGW. GNS in the examined population of RILs ranged from 8 to 42 grains, on average 24. Similar observations for the population K was presented by Milczarski (2010). Mean grain yield per spike of the examined population of RILs amounted to 0.5 g, in the population K - 0.44 g (Milczarski, 2010), and yield per plant (GWP) in the examined population on average for the years of research amounted to 1.6 g, and in the population K - 1.5 g (Milczarski, 2010). Problems with fertility and number of grains per spike in the genotypes of the mapping populations for rice have been described by Xing *et al.* (2002) and for wheat by Kumar *et al.* (2007).

Studies by Zamorska (1968), Wricke (1970), Kaczmarek and Pielas (1970) demonstrate that a decrease in TGW has been observed from the first to the third year of inbreeding. However, according to Słaboński *et al.* (1984), decreasing TGW in inbred lines of rye may be observed also in w S<sub>6</sub>. The inbred lines examined by Słaboński *et al.* (1984) had TGW of 33 g, while inbred lines of rye in a study by Zamorska (1968) 18 - 28g. In the present study on average the lowest TGW values were found for RILs of the examined population in research year 2009. The de-

crease in TGW might have resulted from adverse weather conditions (Miziak *et al.*, 2011). The effect of unfavorable weather conditions (including dry and very hot weather) on the value of this trait has been described in many studies (Słaboński *et al.*, 1984). Unfavorable weather results in fast generation of grains and their worse filling, which causes decreased TGW. In the present study TGW was at the level of 12.6 - 28 g, on average 18.6 g, and in the population K - 17.2 g (Milczarski, 2010).

The population of recombinant inbred lines of rye examined in the present study led to the F<sub>8-10</sub> generation should be considered genetically stable, with segregation within a single locus at the level of 1:1. Exceeding the level of variability found in the population of RILs, exceeding the range of the traits of parental components, may be explained by the transgression effect. The changes of traits observed in the population of RILs, examined in the years, may be accounted for the influence of environmental factors and also fluctuations of the examined traits (Miziak *et al.*, 2011), occurring during inbreeding (F<sub>3-7</sub>), and revealed in the present research as 'continuation through inheritance' of already stabilized effects of segregation of individual loci.

The heterosis effect has been described for F<sub>1</sub> hybrid, obtained in a combination of crossings of lines 153/79-1 × Ot1-3. F<sub>1</sub> hybrid significantly outdid the lines in terms of each trait, which was demonstrated for PT, GNS, SF, GWS, GWP or TGW.

Correlation coefficients for agronomic traits, presented in the present study for RILs of rye, are in accordance with the results of research presented for the same traits by Górný *et al.* (1982), Rzepka-Plevneš *et al.* (1997b), Rzepka-Plevneš and Tomczak (1998), Rzepka-Plevneš and Kulpa (1996) and Milczarski (2010). Significantly positive correlations for the following traits: PH and SL, GNS, TGW, were shown for inbred lines of rye by Górný *et al.* (1982). They amounted to  $r = 0.49^{**}$ ,  $r = 0.28^*$ ,  $r = 0.38^*$ , respectively. For the populations of rye examined in a pot experiment, selected in *in vitro* cultures from open-pollinated varieties of rye, Rzepka-Plevneš *et al.* (1997b) described them respectively as:  $r = 0.23^*$ ,  $r = 0.17^{**}$ ,  $r = 0.49^{**}$ . For the population (S<sub>1</sub>) of rye, selected in hydroponics from open-pollinated varieties of rye, Rzepka-Plevneš and Tomczak (1998), presented them as  $r = 0.36^{**}$ ,  $r = 0.15^*$ ,  $r = 0.43^{**}$ . Rzepka-Plevneš and Kulpa (1996) assessed the correlations for populations (S1 and S2), selected in *in vitro* cultures from open-pollinated varieties of rye, at the level of  $r = 0.03$ ,  $r = 0.03$  and  $r = 0.46^{**}$ , while Milczarski (2010) determined them at the level of  $r = 0.52^{**}$ ,  $r = 0.27^{**}$  and  $r = 0.20^{**}$  for the population K. In the present study the coefficients amounted to  $0.21^{**}$ ,  $0.19^*$  and  $0.37^{**}$ , respectively. Similar correlations were described respectively between: SL and GWS, GWP and TGW in the papers by Górný *et al.* (1982), Rzepka-

Plevneš *et al.* (1997b), Rzepka-Plevneš and Tomczak (1998), Rzepka-Plevneš and Kulpa (1996) and by Milczarski (2010) for the population K. It should be pointed, that the correlation coefficients higher than 0.3 are agronomically important.

Correlation relationships, described between the selected traits of the seedlings of RILs of rye, examined under laboratory conditions, and traits of a mature plant, described in the field experiment, were relatively low and often significant only at  $p < 0.10$ . However, according to the author they confirm the opinions of many authors on relationships between development of traits of seedlings and of a mature plant. For rye they were presented by Górný and Geiger (1982), Górný *et al.* (1982) and Łapiński (1984).

Against a background of the conducted experiment, (significantly) positive correlations between the chosen traits of the seedlings of RILs selected for tolerance to nutrient deficiencies, assessed with the use of morphological criteria of seedlings-the longest root length or root number in *in vitro* cultures of mature embryos (Rzepka-Plevneš and Kulpa, 1996; Rzepka-Plevneš *et al.*, 1997a, b), and traits of a mature plant, described for the population of RILs in the present experiment, may confirm the usefulness of laboratory research for selection of genotypes with increased tolerance to stress caused by nutrient deficiencies (Górný and Szólkowska, 1996; Rzepka-Plevneš and Kulpa, 1996; Rzepka-Plevneš *et al.*, 1997b).

Ward's method of agglomeration on the basis of grouping variables, determining the economical value of a rye inbred line, was used to demonstrate the possibility of formation of extreme groups of the examined RILs - and to select the lines identified also in the extreme groups obtained for the population analyzed under laboratory conditions (Smolik, 2013). As it has been shown, the lines differed significantly in terms of most examined traits.

Each laboratory method has its limitations and in order not to draw too hasty conclusions, the limitations should be taken into account. They were presented only for *in vitro* cultures in a very good review by Hazarika (2006). Additionally, an interesting verification of the obtained results can be carried out after identification of QTLs of the described agronomic traits and comparing them with the results of research presented by Milczarski (2010) and Milczarski *et al.* (2011). What is more, identification of QTLs of biometric traits of seedlings analyzed under both low and high level of nitrogen and potassium can be attempted, and the results can be compared to the QTLs described for root architecture of plants growing under conditions of different abiotic stresses by Tuberosa *et al.* (2002), Mano *et al.* (2006), Zhu *et al.* (2005), Liu *et al.* (2008), including the conditions of nitrogen deficiencies (Tuberosa and Salvi, 2007).

## Conclusions

Inbred lines of rye selected as parental components of the population of RILs differ in most agronomic traits, and the heterosis effect is observed in their  $F_1$  hybrid. A range of variability exceeding the variability described for the traits of parental lines is observed in the population of one hundred thirty-eight RILs. This fact confirms the transgression effect. The existing correlation relationship for agronomic traits of RILs enables selection of lines with definite properties. Positive and often significant correlations between traits of seedlings and selected agronomic traits of a mature plant of examined RILs provide the opportunity for selection of lines tolerant to nutrient stress. Their usefulness for breeding may be assessed only in top-cross experiments or in construction of other mapping populations and verification of obtained QTLs after their previous phenotyping.

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