Electronic Journal of Biotechnology ISSN: 0717-3458
http://www.ejbiotechnology.info
DOI: 10.2225/vol16-issue1-fulltext-5 *RESEARCH ARTICLE*

# Discrimination of population of recombinant inbred lines of rye (*Secale cereale* L.) for different responses to nitrogen-potassium stress assessed at the seedling stage under *in vitro* conditions

**Miłosz Smolik**[1] ✉

[1]West Pomeranian University of Technology, Department of Plant Genetics, Breeding and Biotechnology, Słowackiego, Szczecin, Poland

✉ Corresponding author: msmolik@zut.edu.pl
Received July 7, 2012 / Accepted December 19, 2012
Published online: January 15, 2013


## Abstract

**Background:** Plants differ in the methods used to acquire nutrients from environments with low nutrient availability, and may change the morphology of their 'root architecture' to be able to take up nutrients.
**Results:** In the present study rye response to stress caused by high and low nitrogen-potassium treatments in mature embryos cultures was described within a population consisting of one hundred and thirty eight recombinant inbred lines of rye. Characterization of the response of recombinant inbred lines (RILs) to nutrient stress was presented as the results of analyses of morphological traits, and physiological and biochemical parameters of the seedlings grown in both treatments. A wide range of variability of individual RILs to induced stress was observed in the population of recombinant inbred lines, and was presented as the difference between the means of each of the analysed traits described at high- and low-nitrogen-potassium levels. Lines were grouped using Ward's agglomerative method on the basis of differences in coleoptyle length, with the longest root length and root number used as variables.
**Conclusions:** Recombinant inbred lines at low nitrogen-potassium treatment developed: longer, shorter, or roots of similar length in comparison with the high nitrogen-potassium treatment. Discriminant function analysis showed that the discriminant variable able to clearly differentiate recombinant inbred lines in terms of their response to nutrient stress was the trait of the longest root length.

**Keywords:** mature embryos, nutrient stress, oxidative stress, RILs, rye.

## INTRODUCTION

Rye (*Secale cereale* L.) is an interesting source of genes determining tolerance to edaphic stresses. It has the highest tolerance to drought or nitrogen deficiency, and is more productive than other cereals growing in infertile, sandy, or acid soils, as well as in the poorly-prepared land where it is more often grown (Cakmak et al. 1997; Geiger and Miedaner, 2009; Masojć and Kosmala, 2012).

About 60% of cultivated soils worldwide have plant-growth-limiting problems caused by mineral nutrient deficiencies and toxicity (Lynch, 2007; Liu et al. 2008; Zhang et al. 2010). This situation occurs in Poland, where, for example ca. 60% of the crop production area consists of light-textured or acid soils (Górny and Szołkowska, 1996).

Nitrogen (N) limitation is associated with decreased activity of enzymes that are required for energy metabolism, such as photosynthesis and respiration (Marschner, 1995). Potassium (K) deficiency leads to growth arrest and to impaired sugar and nitrogen balance through inhibition of protein synthesis and long-distance transport (Marschner, 1995; Epstein and Bloom, 2005).

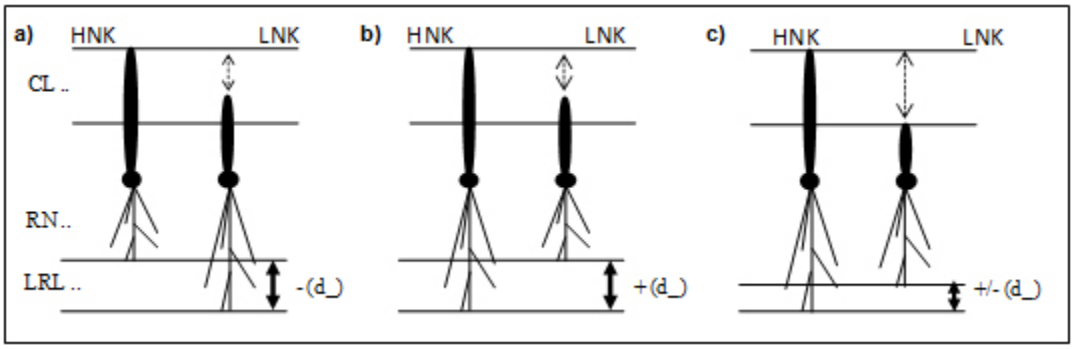


**Fig. 1 The range of variability in the responses of RILs of rye presented as differences (d_) between the mean values of morphological traits of the se edlings grown at HNK and LNK treatments.**

Nitrogen-Potassium interaction (N x K) exists at the morphological, physiological, biochemical and genetically stages during the whole plant growth and generally exists in agricultural ecosystems. K improves the use of N, results in higher yield and quality (Rengel, 2005). K ensures utilization of N and storage of carbohydrates in roots, thus improving NUE (Nutrient-Use Efficiency) (Zhang et al. 2010). Currently, this interaction is a subject of interest in many studies and reviews (Shin et al. 2005; Zhang et al. 2010; Postma and Lynch, 2011; Tsay et al. 2011).

Plants have evolved multiple mechanisms to maintain nutritional homeostasis in diverse edaphic environments (Bloom et al. 1985; Van der Ploeg et al. 1999). Some of these responses can be genetically simple, but most traits (*e.g.* root architecture) are genetically complex (Liu et al. 2008).

Root traits have been shown to play a major role in the adaptive response of crops to low nutrients (Tuberosa et al. 2011; Lynch, 2007). Wiesler and Horst (1994) demonstrated under field conditions that a deeper root system is essential for utilizing nitrate in deep soils, and their selection has often been advocated to mitigate yield losses in crops exposed to nutrient deficits (Górny and Geiger, 1982; Ludlow and Muchow, 1990; Górny, 1995; Krouk et al. 2006).

A morphological change in root elongation and an increase in root number in response to different nutrient status is a 'common' property of many plant species (Zhang and Forde, 1998; Walch-Liu et al. 2006, Zhang et al. 2007; Ruffel et al. 2011). It results from, among other things, the genetic mechanism in plant roots that senses the presence of $NO_3^-$ ions participating as a signalling factor in the signalling pathway of root elongation (Zhang and Forde, 1998). A significant role is played here by the products of the *ANR1* gene (Transcription factors, TFs) and the *AXR4* gene (Zhang et al. 1999). It is still unknown how $NO_3^-$ affects *ANR1*, how *AXR4* acts downstream of *ANR1*, and how the signal is transferred to the genes involved in the process of cell proliferation (Zhang et al. 1999). Remans et al. (2006) suggested that the *AtNTR1.1* gene may act upstream of *ANR1* and could be one of the elements of the transcriptional regulation of *ANR1*, functioning as the nitrate sensor. Gan et al. (2005) identified several genes, out of which seven of the MADS-box genes presented similar expression patterns to *ANR1,* suggesting the existence of undiscovered genes involved in the response to localized nitrate (Zhang et al. 2007).

The application of biotechnology methods in the identification of nutrient-efficient genotypes has an exciting potential. On the one hand, they constitute an alternative method, compared to root evaluation of field-grown plants implemented under controlled conditions (*e.g.* hydroponics, pots or *in vitro*) at an early stage of plant growth (Rzepka-Plevneš et al. 1997a; Sanguineti et al. 2006; Tuberosa et al. 2011; Lynch, 2007). On the other hand, successful regeneration of plants selected under *in vitro* conditions form cell/callus lines proves the usefulness of such methods (Rai et al. 2011).

According to Górny and Szołkowska (1996), Rzepka-Plevneš and Tomczak (1998), Rzepka-Plevneš (1999), Rzepka-Plevneš and Kulpa (1999), Liu et al. (2008) and Messmer et al. (2011), the genotype response to nutrient stress carried out under laboratory conditions at the seedling stage, is reflected in a similar way in the plant maturity stage. However, some authors have different opinions (Bolaños et al. 1993).

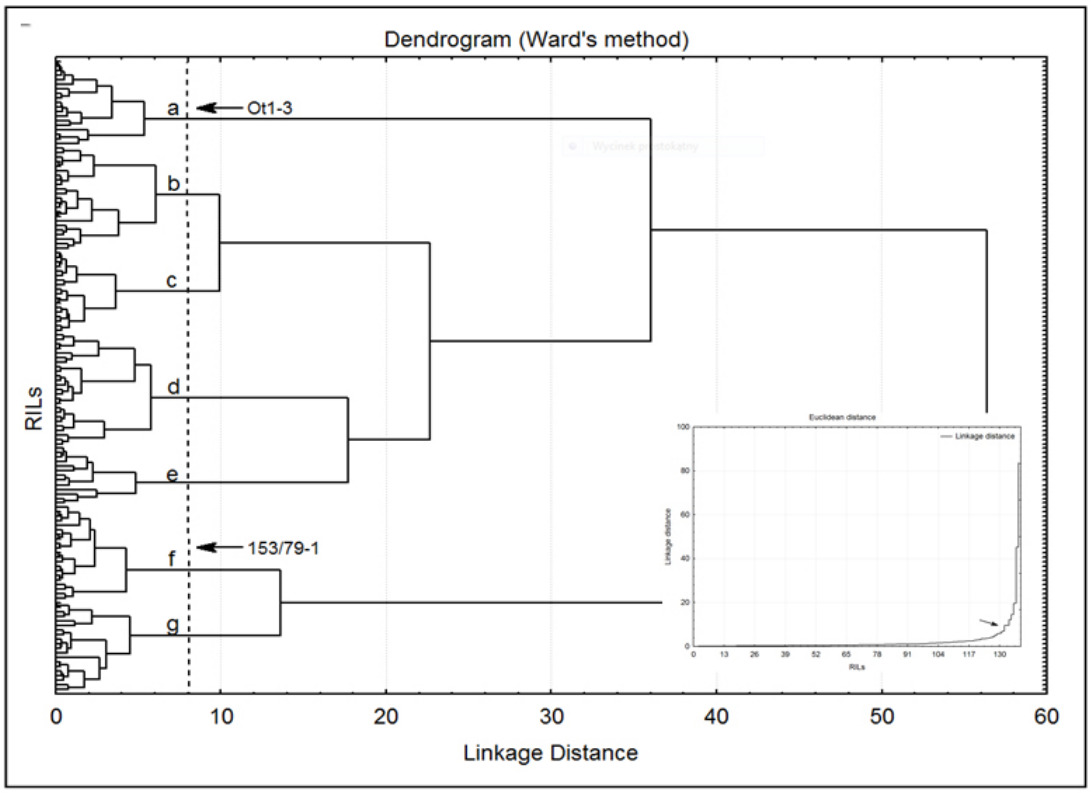


**Fig. 2 Dendrogram of cluster analyses for 138 RILs of rye based on (d_) values assessed as mean difference between CL, LRL and RN for each RIL seedling's grown at HNK and LNK treatments, used as variables using Ward's method.** The vertical lines indicate the cuts-off used to form the groups.

For species of cultivated plants, inbred lines have a wide range of genotypic variability regarding growth at lower mineral concentrations, and selection of tolerant genotypes is carried out depending on the root system (Lynch, 2007; Tuberosa and Salvi, 2007; Löschenberger et al. 2008; Messmer et al. 2011). Bearing in mind that the root is the initial site of nutrient perception, whereas plant root plasticity is a primary effect of the activity of the mechanisms sensing and optimizing consumption and utilization of water and nutrients, the possibility of describing the different responses within segregants in a mapping population enables the investigation of plant responses to low N, K or N-K deprivation; the inhibitory effect of nitrate on root development (Zhang and Forde, 1998; Van der Ploeg, 1999; Tsay et al. 2011; Ruffel et al. 2011); and the possible roles of auxin (Walch-Liu et al. 2006) or ABA (De Smet et al. 2006).

Thus, the aims of this study were to perform phenotype screening and discrimination of population of recombinant inbred lines of rye according to their morphological, physiological and biochemical responses to high- and low- N and K level in medium, to separate the population of RILs into groups differing significantly in terms of their response in order to prepare the tested material for research into the identification of molecular markers linked to QTLs of tolerance to induced stress with the use of the bulked segregant analysis (BSA) method.

## MATERIALS AND METHODS

### Plant population

One hundred and thirty-eight recombinant inbred lines (RILs - $F_7$) of rye (*Secale cereale* L.), deriving from a cross between inbred lines 153/79-1 and Ot1-3, were used in the study. In a preliminary study Rzepka-Plevneš et al. (1997a) have characterized line 153/79-1 as tolerant, whereas Ot1-3 as susceptible to nutrient stress in medium assessed at the seedling stage.

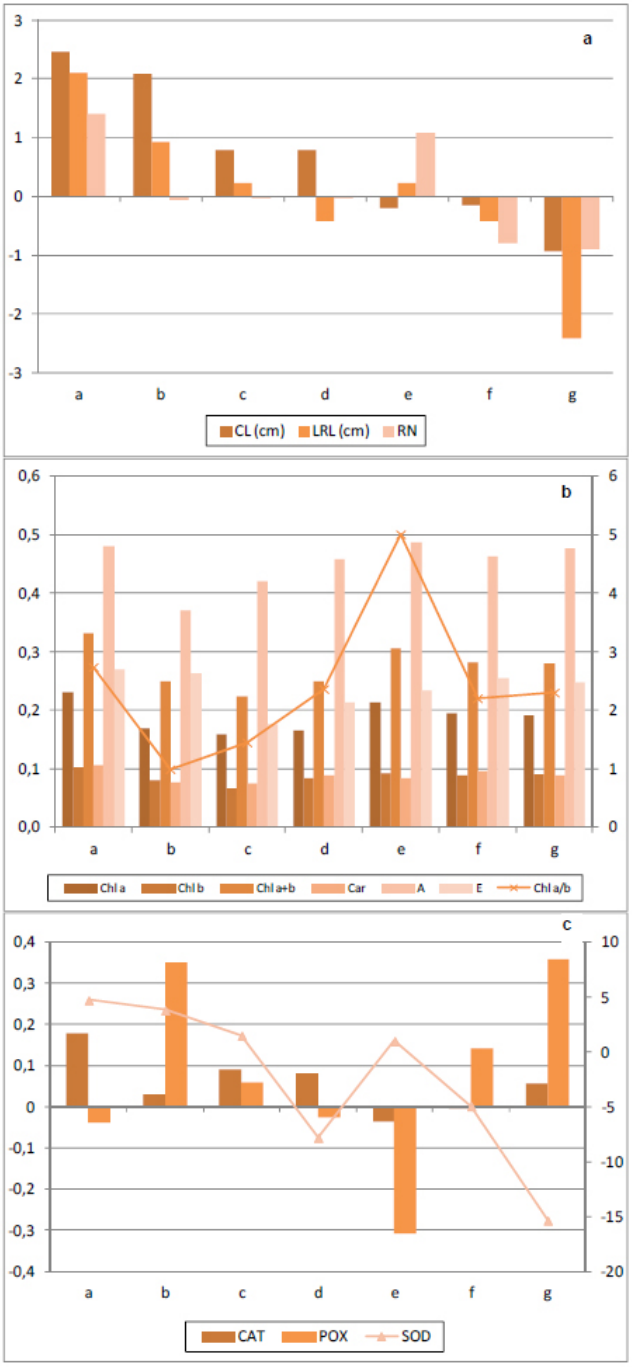


**Fig. 3 The response of recombinant inbred lines of rye to stress caused by N and K deprivation.** (a-c): mean values of differences (d_) for morphological, physiological and biochemical parameters for each group of lines as presented in Figure 2.

Plant embryos culture under *in vitro* conditions and nutrient treatments were establish according to the method described by Rzepka-Plevneš et al. (1997a), Rzepka-Plevneš et al. (1997b) and Rzepka-Plevneš (1999). Rye seeds were surface-sterilized by soaking them for 15 min respectively in 1% and then 7% solution of $H_2SO_4$ and NaOCl. Then they were washed three times for 10 min in distilled water and left in sterile water for 24 hrs. Mature embryos were prepared with the use of a preparation needle, sterilized in 10% NaOCl solution for 10 min, and then washed three times in sterile water and transferred onto appropriate media. Four embryos were placed in each glass tube (9 x 3.5 cm), containing 30 ml of proper media. The tubes were closed with aluminium foil and wrapped with parafilm, than were transferred for 11 days into a phytotron with temperature of 25ºC under cool white light with 16 hrs photoperiod (36 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and 75% RH. The nutrient treatments were composed of MS medium (Murashige and Skoog, 1962). High nitrogen-potassium content (HNK) medium consisted of 6.00 mM N and 2.00 K and used as control, whereas low nitrogen-potassium (LNK) consisted of 0.334 mM of N and 0.333 mM of K. pH of the nutrient media was adjusted to 5.7 (Rzepka-Plevneš et al. 1997a; Rzepka-Plevneš et al. 1997b). A completely randomized design was used with replicates. One glass tube with four seedlings was adopted as one replicate. The glass tubes were evenly laid out in a phytotron with equal light availability. The parental lines and each of the one hundred thirty eight RILs were represented by ca. 150 embryos (ca. 40 glass tubes) and 50 and 100 embryos were placed in each of the media - HNK and LNK, respectively. The plants were harvested after 11 days when they had one (two) visible leaves. At this stage plants grown in LNK became yellow.

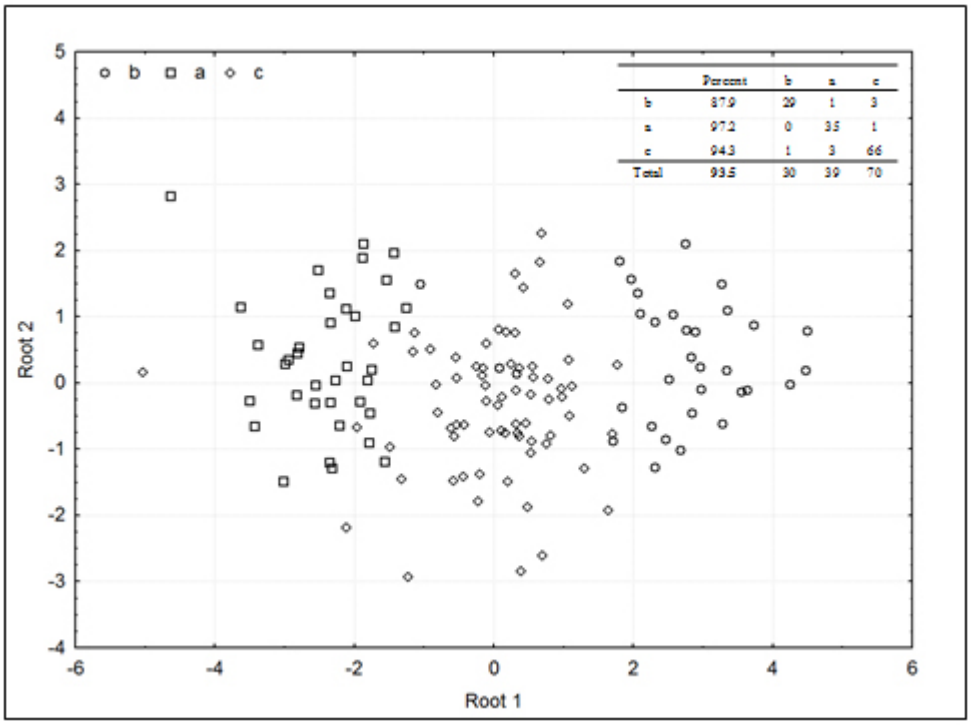


**Fig. 4 Graph of canonical (Root) functions from multivariate discriminant analysis for discrimination by multi-traits analysis between population 138 RILs of rye.** The matrix of RILs classification are presented in the table.

### Seedlings' traits

The seedlings were taken from the agar medium and then washed with distilled water. Biometric measurements, such as: CL (coleoptyle length (cm)), the longest root length (LRL (cm)), and root number (RN), were carried out. Seedlings of each of the tested RILs were taken from the HNK and LNK media respectively, then were pooled and stored at room temperature and -20ºC, respectively, and subjected to physiological and biochemical measurements, respectively. These parameters were assessed each in three replicates for each of the RILs.

**Table 1. Means for seedlings of two inbred lines of rye examined at HNK and LNK with the use of different parameters.**

| Trait | N-K level | Parental lines | | |
|---|---|---|---|---|
| | | 153/79-1 | Ot1-3 | |
| CL (cm) | HNK | 10.56 ± 1.58 | 7.15 ± 0.89 | * |
| | LNK | 9.33 ± 1.12 | 5.27 ± 0.64 | * |
| | | *ns* | *ns* | |
| LRL (cm) | HNK | 6.69 ± 0.97 | 4.7 5 ± 0.65 | * |
| | LNK | 7.45 ± 1.25 | 2.25 ± 0.64 | * |
| | | *ns* | ** | |
| RN | HNK | 5.19 ± 1.18 | 5.18 ± 0.89 | *ns* |
| | LNK | 5.19 ± 1.04 | 3.71 ± 0.78 | *ns* |
| | | *ns* | * | |
| Chl *a* | HNK | 0.41 ± 0.02 | 0.71 ± 0.04 | * |
| (mg · $g^{-1}$ FW) | LNK | 0.38 ± 0.03 | 0.46 ± 0.06 | *ns* |
| | | *ns* | * | |
| Chl *b* | HNK | 0.17 ± 0.02 | 0.30 ± 0.03 | * |
| (mg · $g^{-1}$ FW) | LNK | 0.17 ± 0.05 | 0.19 ± 0.04 | *ns* |
| | | *ns* | * | |
| Chl *a+b* | HNK | 0.58 ± 0.05 | 1.01 ± 0.06 | * |
| (mg · $g^{-1}$ FW) | LNK | 0.54 ± 0.04 | 0.65 ± 0.07 | *ns* |
| | | *ns* | ** | |
| Chl *a/b* | HNK | 2.44 ± 0.14 | 2.42 ± 0.15 | *ns* |
| | LNK | 2.28 ± 0.20 | 2.43 ± 0.23 | *ns* |
| | | *ns* | *ns* | |
| Car | HNK | 0.23 ± 0.02 | 0.39 ± 0.04 | * |
| (mg · $g^{-1}$ FW) | LNK | 0.21 ± 0.02 | 0.25 ± 0.06 | *ns* |
| | | ** | * | |
| A | HNK | 1.68 ± 0.13 | 1.97 ± 0.29 | ** |
| (µmol $CO_2$ · $m^{-2}$ · $s^{-1}$) | LNK | 1.12 ± 0.14 | 1.52 ± 0.25 | ** |
| | | ** | ** | |
| *E* | HNK | 0.57 ± 0.06 | 1.03 ± 0.06 | ** |
| (mmol $H_2O$ · $m^{-2}$ · $s^{-1}$) | LNK | 0.27 ± 0.07 | 0.34 ± 0.05 | ** |
| | | ** | ** | |
| CAT | HNK | 0.63 ± 0.02 | 0.63 ± 0.02 | *ns* |
| (µmol $H_2O_2$· $g^{-1}$ FW · $min^{-1}$) | LNK | 0.76 ± 0.02 | 0.27 ± 0.06 | * |
| | | *ns* | * | |
| POX | HNK | 1.64 ± 0.16 | 1.99 ± 0.16 | *ns* |
| (µmol purpurogalin · $g^{-1}$ FW · $min^{-1}$) | LNK | 1.90 ± 0.12 | 1.51 ± 0.25 | * |
| | | * | * | |
| SOD | HNK | 260 ± 15.31 | 186 ± 10.01 | ** |
| (U· $g^{-1}$ FW) | LNK | 241 ± 13.76 | 160 ± 19.31 | ** |
| | | *ns* | * | |

Each datum represents mean ± SD (standard deviation); *ns:* not significant; *P < 0.05; **P < 0.01 significant differences between inbred lines. Significance of differences between the means was calculated with Tukey's t-test.

### Photosynthetic pigments, assimilation and transpiration

After 11 days of treatment, seedling samples were taken for the assessment of chlorophyll concentration (mg $g^{-1}$ FW), according to Lichtenthaler and Wellburm (1983), and of carotenoid (Car) content, according to Hager and Meyer-Berthenrath (1966). Total chlorophyll [Chl (*a* + *b*)], chlorophyll a (Chl *a*), chlorophyll b (Chl *b*) and Car contents were determined. $CO_2$ assimilation rate (A) and transpiration rate (*E*) were measured using a TPS-2 gas analyser by PP Systems (UK) according to the standard method described by Von Caemmerer and Farquhar (1981).

Total catalase (CAT, EC 1.11.1.6) activity was measured spectrophotometrically (UV/VIS Lambda Bio, Perkin-Elmer) according to the method by Lück (1965) by monitoring a decline in absorbance at λ = 240 nm for 60 sec as $H_2O_2$ was consumed. Total peroxidase (POX, EC 1.11.1.7) assay contained 20 mM pyrogallol, 1 mM $H_2O_2$, and 50 ml of plant extract in 50 mM potassium phosphate buffer (pH 7.0) in a total volume of 1 ml (Chance and Maehly, 1955). Purpurogallin formation was measured spectrophotometrically (Nova 400, Merck) at λ = 430 nm at 25ºC for 4 min. Total superoxide dismutase (SOD, EC 1.11.1.5) activity was analysed according to Abassi et al. (1998). Increasing volumes (25,

50, 100 and 200 µl) of leaf extracts were added to the reaction mixture to the final volume of 3 ml. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.8), 13 mM L-methionine, 0.1 mM EDTA, 2 µM riboflavin and 75 µM nitroblue tetrazolium (NBT).

The experiment was carried out in stages by characterization of 8-10 subsequent RILs in every stage in terms of all the mentioned parameters. In the case of contamination of rye embryo cultures, single contaminated glass tubes were removed. When more embryos were contaminated, the experiment was repeated for a given line.

**Table 2. Descriptive statistics of the morphological, physiological and biochemical parameters and two-way variance analysis of seedling traits of the RILs of rye grown at HNK and LNK treatments.**

| Trait | N-K | RILs | | | | | | Analysis of variance | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | level | Min | Max | Mean ± SD | CV% | Bias | Kurtosis | NK | RILs | (NKxG) |
| CL (cm) | HNK | 2.89 | 11.01 | 6.47 ± 1.18 | 15.58 | 0.33 | -0.35 | ** | ** | ** |
| | LNK | 2.89 | 10.00 | 5.42 ± 1.13 | 17.40 | -0.04 | -0.86 | | | |
| LRL (cm) | HNK | 1.18 | 6.69 | 3.21 ±0.96 | 28.81 | -0.79 | 1.65 | ** | ** | ** |
| | LNK | 0.64 | 7.45 | 3.43 ±1.28 | 36.60 | -0.52 | 0.35 | | | |
| RN | HNK | 1.64 | 5.37 | 3.77 ±0.61 | 17.36 | 0.03 | 1.29 | ** | ** | ** |
| | LNK | 1.87 | 5.20 | 3.66 ± 0.64 | 19.06 | -0.17 | -0.37 | | | |
| Chl *a* (mg · $g^{-1}$ FW) | HNK | 0.29 | 1.06 | 0.60 ± 0.13 | 20.93 | 0.48 | 1.08 | ** | ** | ** |
| | LNK | 0.14 | 0.66 | 0.42 ± 0.09 | 21.06 | 0.02 | 0.40 | | | |
| Chl *b* (mg · $g^{-1}$ FW) | HNK | 0.14 | 0.49 | 0.28 ± 0.06 | 21.09 | 0.67 | 1.39 | ** | ** | ** |
| | LNK | 0.09 | 0.46 | 0.19 ± 0.05 | 24.84 | 1.38 | 6.08 | | | |
| Chl *a+b* (mg · $g^{-1}$ FW) | HNK | 0.45 | 1.55 | 0.88 ± 0.18 | 20.22 | 0.58 | 1.38 | ** | ** | ** |
| | LNK | 0.23 | 1.08 | 0.61 ± 0.12 | 20.26 | 0.23 | 1.26 | | | |
| Chl *a/b* | HNK | 1.28 | 3.81 | 2.17 ± 0.28 | 12.71 | 1.28 | 1.99 | ** | ** | ** |
| | LNK | 1.25 | 3.91 | 2.20 ± 0.45 | 20.24 | 1.27 | 2.98 | | | |
| Car (mg · $g^{-1}$ FW) | HNK | 0.14 | 0.46 | 0.27 ± 0.05 | 19.73 | 0.39 | 0.69 | ** | ** | ** |
| | LNK | 0.07 | 0.30 | 0.19 ± 0.04 | 20.91 | 0.17 | 0.22 | | | |
| A (µmol $CO_2$ · $m^{-2}$ · $s^{-1}$) | HNK | 1.31 | 2.89 | 1.88 ± 0.29 | 15.40 | 0.75 | 0.97 | ** | ** | ** |
| | LNK | 0.91 | 2.31 | 1.44 ± 0.22 | 15.58 | 0.86 | 2.33 | | | |
| *E* (mmol $H_2O$ · $m^{-2}$ · $s^{-1}$) | HNK | 0.21 | 1.02 | 0.56 ± 0.17 | 30.61 | 0.54 | -0.28 | ** | ** | ** |
| | LNK | 0.15 | 0.95 | 0.32 ± 0.12 | 37.25 | 1.92 | 6.19 | | | |
| CAT (µmol $H_2O_2$· $g^{-1}$ FW · $min^{-1}$) | HNK | 0.16 | 1.28 | 0.57 ± 0.20 | 35.27 | 0.56 | 0.76 | ** | ** | ** |
| | LNK | 0.20 | 1.12 | 0.51 ± 0.18 | 34.78 | 0.82 | 1.12 | | | |
| POX (µmol purpurogalin · $g^{-1}$ FW · $min^{-1}$) | HNK | 0.20 | 3.21 | 1.10 ± 0.69 | 62.44 | 1.27 | 1.19 | ** | ** | ** |
| | LNK | 0.27 | 3.10 | 0.99 ± 0.65 | 65.70 | 1.60 | 2.29 | | | |
| SOD (U· $g^{-1}$ FW) | HNK | 135.89 | 257.92 | 184.83±24.48 | 13.25 | 0.72 | 0.36 | ** | ** | ** |
| | LNK | 134.98 | 275.33 | 187.86±27.69 | 14.74 | 0.54 | 0.28 | | | |

SD: standard deviation; *ns:* not significant; *P < 0.05; **P < 0.01; significant differences between RILs.

## Statistics

Two-way analysis of variance was carried out for the traits of the parent lines, 138 RILs and treatments (HNK and LNK). Changes of root elongation under nutrient stress are a typical plant adaptation mechanism and depend on genotype and initiated defense strategy. Response of each of the one hundred thirty eight RILs and parent lines to nutrient stress caused by N and K deficiency has been presented as value of difference (d_) between means for the seedlings growing at HNK and LNK (Figure 1). Their significance was tested using the Student's *t*-test. For each RIL the value of difference (d_) between means was positive or negative, depending on examined trait and RIL response. The differences (d_) between the means for three seedling traits: d_CL, d_LRL and d_RN were used to carry out cluster analysis with the use of Ward's agglomerative method (Euclidean distances). Grouping variables ('a' and 'b') were assigned to the RILs grouped in the groups of Ward's dendrogram together with parental lines, while variable 'c' to other RILs. The groups were characterized according to investigated parameters. Discriminant function analysis (DFA) was performed in order to answer the question, whether other physiological or biochemical parameter, apart from seedling traits - *i.e.* CL, LRL or RN, are also important in discrimination of RILs' population. The parental lines were added to

such proposed a *posteriori* division of the 138 RILs into three groups. The verification of the traits by forward selection method in the DFA was based on the significance of Wilk's $\lambda$ test, approximate value of *F* test. The significance level corresponding to the *F*-value for entering or staying on a specific trait at each step was set at $P = 0.20$. All traits that remained in the model, when the stepwise selection process stopped, were considered to discriminate significantly between groups. Also, coefficients of canonical variables after their standardization were calculated, as well as accumulated percentage of explained variability in a given model. The assessment of model significance was verified with the use of $\chi^2$ test. The ability of the created models to divide RILs into groups was illustrated on a chart of scatter of canonical values. The above-presented calculations were made using Statistica 9 software package (StatSoft PL).

## RESULTS

### Phenotyping

A wide range of variability in the response of the examined rye genotypes to nutrient stress caused by the LNK treatment was found both for the parental lines and within the population of the 138 RILs ($F_7$). The mean values of seedling CL, LRL and RN, physiological (Chl *a*, *b*, *a+b*, *a/b*, Car, A, *E*) and biochemical (total CAT, SOD and POX) parameters assessed at the HNK and LNK treatments for both parental lines and 138 RILs are presented in Table 1 and Table 2.

It was concluded that inbred lines responded to induced stress (LNK) in different ways. Under HNK conditions they generally generated longer coleoptyles in comparison to LNK. Line 153/79-1 developed significantly longer LRL at LNK than at HNK. Line Ot1-3 responded in a different way, and the differences were described as significant (Table 1). For RN of line 153/79-1, no significant differences were noted between HNK and LNK. Differences were found for line Ot1-3 (Table 1). When other physiological parameters were considered, it was demonstrated that under HNK conditions, the parental lines generally had greater values of the assessed parameters in comparison with LNK. The differences were not always significant. They are presented in detail in Table 1. Line Ot1-3 had significantly lower biochemical parameters at LNK in comparison to HNK. A similar response was found for line 153/79-1, and a significant difference was described only for POX activity (Table 1).

Different seedling parameter values resulted from not only the experimental conditions but also from genotype. Line 153/79-1, as opposed to Ot1-3, was characterized by longer coleoptyle, longer LRL and more numerous roots (Table 1). In terms of both physiological and biochemical parameters, genotypic differences between the lines had different values. They are presented in detail, together with the significance of the differences in Table 1.

Among the RILs significant differences were found in all the examined seedling traits and in all physiological and biochemical parameters in both NK treatments (Table 2).

Variance analysis showed that the effects of NK treatments, genotype and NK x genotype interaction were highly significant for all the seedling traits, as well as for physiological and biochemical parameters (Table 2). In HNK and LNK treatments some RILs generally developed shorter coleoptyles, longer roots and a greater number of roots. Also, on average lower concentrations of Chl *a, b* and Car and lower A and *E* as well as decreased of CAT and POX activity and increased SOD activity were found (Table 2).

In both NK treatments highly significant correlations between CL, LRL and RN were found (Table 3). Similarly, significant and highly significant correlations were assessed between SOD and CL, and LRL and RN, respectively. Highly significant correlations between photosynthetic pigments, A and *E*, were also found in both NK treatments (Table 3). In HNK, a significant correlation between the activity of CAT and POX was observed, and this was not found for the LNK treatment (Table 3).

It has been demonstrated that RILs developed longer CL at HNK when compared to LNK, and some RILs developed significantly longer LRL and more numerous roots (RN) at HNK in comparison to LNK (Figure 1a). The other RILs developed significantly shorter LRL and less numerous roots (RN) at HNK in comparison to LNK (Figure 1b).

According to the calculations performed with the use the of Student *t*-test, some of the differences (d_) were significant, highly significant or non-significant, for which differences between means (d_) of individual traits were at a similar level (data not shown) (Figure 1c). The range of variability for individual parameters of RILs is presented in Table 4 as difference (d_) and standard deviation values (SD), and also as minimum and maximum values. It was demonstrated that at HNK the examined RILs on average developed longer coleoptyles (d_CL = 0.74), shorter LRL (d_LRL = -0.24) and a similar number of roots (d_RN = 0.01). For the other parameters mean differences (d_) for the whole population had positive values, which proved that a decrease in their values was observed for individual RILs. The SOD parameter was an exception - a greater activity of the enzyme was found at LNK in comparison to HNK (-3.03) (Table 4 and Figure 1).

**Table 3. Correlation coefficients among rye seedling's traits and selected physiological and biochemical parameters at LNK and HNK treatments.**

| | | CL | LRL | Chl *a* | Chl *b* | Chl *a+b* | Chl *a/b* | Car | A | *E* | CAT | POX | SOD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| RN | HNK | 0.45** | 0.53** | 0.04 | 0.01 | 0.03 | 0.05 | 0.09 | -0.01 | 0.07 | 0.02 | -0.13 | 0.30* |
| | LNK | 0.62** | 0.65** | 0.07 | -0.08 | 0.02 | 0.17* | -0.03 | 0.13 | 0.13 | -0.13 | -0.20* | 0.32* |
| CL | HNK | | 0.61** | 0.05 | -0.04 | 0.02 | 0.14 | 0.11 | -0.14 | 0.03 | -0.06 | -0.05 | 0.57** |
| | LNK | | 0.64** | 0.04 | -0.13 | -0.02 | 0.16 | 0.04 | -0.05 | -0.04 | -0.08 | -0.22 | 0.51** |
| LRL | HNK | | | 0.00 | -0.01 | 0.00 | 0.01 | 0.04 | -0.18* | 0.02 | 0.00 | -0.03 | 0.31* |
| | LNK | | | 0.00 | -0.13 | -0.05 | 0.13 | -0.05 | 0.06 | 0.04 | -0.13 | -0.14 | 0.41* |
| Chl *a* | HNK | | | | 0.84** | 0.98** | 0.26* | 0.86** | 0.44** | 0.49** | 0.03 | -0.09 | 0.05 |
| | LNK | | | | 0.63** | 0.95** | 0.37* | 0.78** | 0.27* | 0.29* | 0.11 | -0.09 | -0.03 |
| Chl *b* | HNK | | | | | 0.92** | -0.28* | 0.86** | 0.42* | 0.36* | -0.02 | -0.07 | -0.05 |
| | LNK | | | | | 0.83** | -0.46** | 0.72** | 0.14 | 0.01 | 0.08 | 0.12 | -0.14 |
| Chl *a+b* | HNK | | | | | | 0.09 | 0.89** | 0.45** | 0.46** | 0.02 | -0.08 | 0.02 |
| | LNK | | | | | | 0.08 | 0.84** | 0.25* | 0.21* | 0.11 | -0.02 | -0.08 |
| Chl *a/b* | HNK | | | | | | | 0.01 | 0.01 | 0.18* | 0.08 | -0.03 | 0.14 |
| | LNK | | | | | | | 0.00 | 0.12 | 0.33* | 0.05 | -0.22 | 0.11 |
| Car | HNK | | | | | | | | 0.43** | 0.42** | 0.00 | 0.00 | 0.05 |
| | LNK | | | | | | | | 0.23* | 0.13* | -0.01 | 0.01 | -0.02 |
| A | HNK | | | | | | | | | 0.50** | -0.10 | 0.04 | 0.02 |
| | LNK | | | | | | | | | 0.51** | -0.09 | 0.02 | 0.05 |
| *E* | HNK | | | | | | | | | | 0.19 | 0.08 | 0.00 |
| | LNK | | | | | | | | | | 0.00 | 0.01 | 0.06 |
| CAT | HNK | | | | | | | | | | | 0.28* | -0.12 |
| | LNK | | | | | | | | | | | 0.07 | 0.02 |
| POX | HNK | | | | | | | | | | | | -0.08 |
| | LNK | | | | | | | | | | | | -0.05 |

*P < 0.05; **P < 0.01 significantly different from zero.

## Clustering

Cluster analysis of the set of RILs and two parent lines based on the d_CL, d_LRL and d_RN indicated that all the genotypes were grouped in seven discrete groups (Figure 2).

Groups 'a' and 'c' consisted of eighteen RILs each. Groups 'b' and 'd' included twenty-three and twenty-five RILs, while groups 'e', 'f' and 'g' - twelve, twenty-one and twenty (Figure 3). In general, the RILs in groups 'a' and 'b' exhibited a significantly shorter CL, LRL and less RN in the LNK in comparison to the HNK, whereas the RILs in groups 'f' and 'g' were comprised of genotypes expressing a significantly longer CL, LRL and more RN in the LNK in comparison to the HNK (Figure 3). The characteristics of d_CL, d_RN, and especially of d_LRL between groups 'a' and 'g' showed the possibility of discrimination of the whole population according to their different responses to nutrient stress and their classification into extreme groups: susceptible ('a') and tolerant ('g'), while lines with similar responses were in the groups - 'b' and 'f' (Figure 3a). Figure 3b and Figure 3c present the physiological and biochemical responses of the RILs, which were classified as shown in Figure 3a. Positive values of (d_) for individual physiological parameters, proving their decrease at the LNK in comparison with the HNK, were found in each of the groups. Assessment of CAT, POX and SOD activity in the individual groups demonstrated that differences between the means were positive and/or negative (Figure 4c). A decrease in the activity of CAT and SOD and a slight increase in POX were

observed under the LNK treatment in group ‘a’, while in group ‘g’ a decrease in CAT and POX and a distinct increase in SOD activity were noted (Figure. 3c).

**Discriminant function analysis and canonical analysis**

After application of forward stepwise DFA to the data (138 RILs x 13 variables), the variables presented in Table 5 were retained in the model. A highly significant statistical value of the Wilk’s $\lambda$ test = 0.214; $F$ = 16.530; $P$ < 0.0000 $F$ was demonstrated; hence the discrimination of RIL response to nutrient stress was highly significant. Nine variables (d_LRL, d_CAT, d_POX, d_Car, d_Chl *b*, d_SOD, d_A, d_Chl *a* and d_*E*) were used in the model of discriminant function analysis (Table 5).

The greatest observable contribution came from d_LRL ($\lambda$ = 0.333, $F$ = 128.020), followed by d_CAT ($\lambda$ = 0.932, $F$ = 4.650) and d_Car ($\lambda$ = 0.937; $F$ = 4.322). The other variables, despite their inclusion in the model, turned out to be non-significant in predicting affiliations to rye groups tolerant or susceptible to nutrient stress. Eigenvalues and the corresponding standardized canonical discriminant function coefficients are presented in Table 5. The first function (Root 1) generated the eigenvalue of 3.13, while the second one gave 0.131 (Root 2). The variance calculated for the first function, and the percentage of the total explained variance expressed in the first function was 95.98 (Table 5). The highest standardized discriminant coefficients in Root 1 originated from: d_LRL (1.004), d_Car (-0.624) and d_Chl *b* (0.316), whereas in Root 2 they were from: d_Car (-0.870), d_CAT (0.782), and d_Chl *a* (0.541). Both functions were statistically significant (Root 1: R-canon = 0.871; Wilk’s $\lambda$ = 0.214; $\chi^2$ = 203.59; $P$ < 0.0000; Root 2: R-canon = 0.340; Wilk’s $\lambda$ = 0.884; $\chi^2$ = 16.26; $P$ < 0.038); and the first function was responsible for ca. 96% of the explained variance. A two-dimensional scatter plot using the discriminant scores of the RILs along Roots 1 and 2 indicates a satisfactory separation of the recombinant inbred lines according to their response to nutrient stress assessed under *in vitro* conditions at the seedling stage (Figure 4).

Figure 4 presents the classification matrix of the RILs, from which it appears that 93.5% of the RILs were correctly classified into groups ‘a’, ‘b’ and ‘c’ with the use of the calculated classification functions.

**DISCUSSION**

The present study demonstrated the usefulness of mature rye embryos cultures, in research on nutrient stress according to the method described by Rzepka-Plevneš et al. (1997a), Rzepka-Plevneš et al. (1997b), Rzepka-Plevneš, (1999) and Rzepka-Plevneš and Kulpa (1999).

They have also been used in many other experiments, including for describing the response of rice to drought (Joshi et al. 2011) or tolerance to saline and heat stresses in wheat (Benderradji et al. 2012). Rakoczy-Trojanowska and Malepszy (1995) found significant differences between the *in vitro* response of several inbred rye lines, both in the case of immature inflorescences and immature embryos.

Elicitation of the response potential *per se* of each of the 138 investigated recombinant inbred lines of rye to stress caused by nitrogen and potassium deficiencies was obtained by analysis of morphological changes in CL, LRL and RN in the seedlings growing in the HNK and LNK treatments. The state, in which the examined rye seedlings generally developed shorter CL in the LNK treatment in comparison with the HNK, may be the result of changes in root-to-shoot ratio, and can be explained on the one hand by the functioning of the mechanism of root elongation described by Zhang et al. (2007), or on the other by allocation of assimilates from the shoot to the root, taking place in plants under nutrient stress (Bloom et al. 1985; Cai et al. 2012). According to Mi et al. (2007) N-efficient maize inbred lines allocate more N to root development, and can develop more root compared to inefficient genotypes.

It was demonstrated that CL such as LRL and RN - elements of ‘root architecture’, had different values in individual RILs. The traits depend on genotype (Messmer et al. 2011). Despite the fact that CL in the parental lines was not affected by the NK treatments, a significant influence of genotypic differences was found. In the inbred lines LRL was significantly affected by both genotype and the treatments. The seedlings of line 153/79-1 developed longer LRL at LNK than at HNK, while the converse occurred with seedlings of line Ot1-3 - they developed on average shorter LRL, and the differences were highly significant. Neither genotypes in the both parental lines nor the treatments for line 153/79-1 had any

influence on the differences in RN. Line Ot1-3 developed a lower number of roots in the LNK (Table 1). On average longer CLs (6.47 cm) were noted for the whole population of the RILs tested in the HNK treatment in comparison with the LNK (5.42 cm). Longer LRLs were described for the RILs tested in the LNK (on average 3.43 cm) in comparison to the HNK (3.21 cm); similarly, a greater RN was noted in the RILs tested in the LNK. A similar response from maize seedlings affected by both low N and high N was presented by Wang et al. (2005) and Liu et al. (2008). Different responses from the seedlings of individual RILs compared to parental lines may be explained by genotypic differences and possible transgression, which was described by Górny (1995) for barley, by Górny and Szołkowska (1996) for oat and by Liu et al. (2008) for maize.

**Table 4. Differences (d_) between mean values of traits, basis and kurtosis, demonstrating range of variability and response to nutrient stress caused by NK treatments for RILs and parental lines of rye.**

| Trait | RILs | | | | | Parental lines | |
|---|---|---|---|---|---|---|---|
| | Min | Max | Mean | Bias | Kurtosis | 153/79-1 | Ot1-3 |
| d_CL (cm) | -2.93 | 3.98 | 0.74 ± 1.11 | 0.18 | 0.21 | 1.23 ± 0.27 | 1.88 ± 0.28 |
| d_LRL (cm) | -5.06 | 3.99 | -0.24 ± 1.48 | 0.32 | -0.14 | -0.76 ± 0.36 | 2.50 ± 0.26 |
| d_RN | -2.19 | 2.43 | 0.01 ± 0.74 | 0.17 | 0.73 | 0.00 ± 1.00 | 1.47 ± 0.31 |
| d_Chl *a* (mg · $g^{-1}$ FW) | -0.19 | 0.76 | 0.19 ± 0.14 | 0.76 | 2.54 | 0.25 ± 0.05 | 0.03 ± 0.02 |
| d_Chl *b* (mg · $g^{-1}$ FW) | -0.18 | 0.35 | 0.09 ± 0.07 | 0.24 | 2.82 | 0.11 ± 0.05 | 0.11 ± 0.02 |
| d_Chl *a+b* | -0.29 | 1.11 | 0.27 ± 0.10 | 0.75 | 2.57 | 0.36 ± 0.10 | 0.04 ± 0.05 |
| d_Chl *a/b* | -27.00 | 26.00 | 2.19 ± 1.94 | 2.42 | 1.78 | -0.01 ± 0.24 | 0.16 ± 0.01 |
| d_Car (mg · $g^{-1}$ FW) | -0.10 | 0.33 | 0.09 ± 0.06 | 0.50 | 1.89 | 0.14 ± 0.05 | 0.02 ± 0.02 |
| d_A (µmol $CO_2$ · $m^{-2}$ · $s^{-1}$) | 0.00 | 1.30 | 0.45 ± 0.19 | 0.80 | 0.30 | 0.45 ± 0.05 | 0.56 ± 0.06 |
| d_*E* (mmol $H_2O$ · $m^{-2}$ · $s^{-1}$) | -0.07 | 0.64 | 0.24 ± 0.14 | 0.53 | 0.26 | 0.69 ± 0.06 | 0.30 ± 0.09 |
| d_CAT (µmol $H_2O_2$· $g^{-1}$ FW · $min^{-1}$) | -0.64 | 0.61 | 0.06 ± 0.21 | 0.30 | 0.42 | -0.13 ± 0.05 | 0.35 ± 0.04 |
| d_POX (µmol purpurogalin · $g^{-1}$ FW · $min^{-1}$) | -2.55 | 2.61 | 0.11 ± 0.76 | 0.39 | 2.37 | -0.26 ± 0.09 | 0.48 ± 0.08 |
| d_SOD (U· $g^{-1}$ FW) | -32.58 | 23.90 | -3.03 ± 14.8 | 0.11 | -1.08 | 19 ± 5.85 | 26.0 ± 3.61 |

A morphological change of in root elongation and an increase in root number is a 'common' response of many plant species to different nutrient status (Zhang and Forde, 1998; Walch-Liu et al. 2006). It may be assumed that the RILs which developed shorter roots in the LNK treatment in comparison with the HNK, may have genes changing the mechanism of 'root elongation' by specific TFs 'modulating' or silencing the expression of *e.g. ANR1* (Zhang and Forde, 1998; Walch-Liu et al. 2006). Inhibition of root elongation is in accordance with the 'dormant strategy' (Chun et al. 2005; Guo et al. 2005). A possible explanation for the observed reaction in the selected RILs may be the inhibition of their root elongation resulting from initiation of the mechanism sensing N nutritional status of a plant as extremely low. Such a response type was described as differentiating inbred maize lines by Chun et al. (2005) and Guo et al. (2005).

Walch-Liu et al. (2006) suggest that up- and down-regulation of the *ANR1* gene under conditions of low and high N status (not necessarily of $NO_3^-$) can be viewed as a mechanism modulating the intensity of lateral root response to localized N supply. Authors state that the molecular mechanism of plant response to N stress may involve (apart from *ANR1*) dozens of genes of the MADS-box gene family (TFs).

The low concentration of nitrogen and potassium in the medium could have resulted in increased pressure under laboratory conditions for the genotypes responding to nutrient stress (-N-K) in different ways. Research on the transcriptome of model plants indicates that in general plant response to different abiotic factors, including N and K deficiencies, often has a common genetic background (Ruffel et al. 2011).

Cakmak and Engels (1999) and Zhang et al. (2010) state that very low rates of potash fertilizer application result in ineffective utilization of the applied nitrogen fertilizers. Shin et al. (2005) showed that changes in ROS concentrations in wild-type *Arabidopsis* and in a set of root hair mutants suggest that the hair root cells are important for the response to nitrogen and potassium starvation. K starvation induces the expression of *AtNRT1.5* and increases the activity of the GS (glutamine synthetase)/GOGAT (glutamate synthase)/GDH (glutamate dehydrogenase) cycle in K-starved roots. The latest research has shown that kinase CIPK23 (CBL-interacting protein kinase 23) is involved in regulating both $K^+$ and $NO_3^-$ uptake (Tsay et al. 2011).

The response of the examined rye RILs to nutrient stress caused by different concentrations of N and K in HNK and LNK treatments is in accordance with the results of the study presented by Rzepka-Plevneš (1999) for open-pollinated, selected populations or breeding strains of rye. Rzepka-Plevneš (1999) conducted triple mass selection among seedlings of eleven rye populations and two strains, regenerated from mature embryos *in vitro* culture by assessment of CL, LRL and RN. The author distinguished genotypes tolerant to nutrient deficiencies in medium, which, as later demonstrated in the author's field experiment, did not differ significantly from the control in terms of the selected parameters of yield (Rzepka-Plevneš and Kulpa, 1999). This results support the opinion presented by Rai et al. (2011) on the usefulness of the method for preliminary evaluation/selection of response to abiotic stress of mature embryos culture conducted for different genotypes of rye and other cereals.

Similar results for seedlings grown in nutrient stress in hydroponics (N control - 10 mM, low-N - 1.67 mM) for parents of oat and their progenies ($F_5$) were presented by Górny and Szołkowska (1996), for rye seedlings by Rzepka-Plevneš and Tomczak (1998), for maize seedlings treated by low and high N by Wang et al. (2005) and Liu et al. (2008).

Significant correlations between RN and CL, RN and LRL, and CL and LRL were reported. The results are in accordance with the results presented for inbred lines of rye and their hybrids by Górny and Geiger (1982) and Rzepka-Plevneš (1999). Górny and Geiger (1982) presented (highly) significant correlations for inbred lines of rye and their hybrids between: root number (RN) and total root system length (TRSL); RN and total root system weight (TRSW); the longest root length (LRL) and TRSL; LRL and TSRW; and TRSL and TRSW. The authors reported high heritability coefficients for root traits of rye LRL ($h^2$ = 0.50) and shoot fresh weight ($h^2$ = 0.75). Similar results were obtained in the present study. Highly significant correlations were found for RN and CL, RN and LRL, and CL and LRL. In another paper Górny et al. (1982) demonstrated significant correlations between rye traits, such as: LRL, TRSL, TRSW and grain number (GN), 1000-grain weight (TGW), grain yield per spike (GYS), assessed for seedlings (under laboratory conditions) and adult plants (micro-plot data). Moreover, the authors showed highly significant correlations between LRL and plant height (0.41) and grain yield per ha (0.51), assessed under laboratory conditions, and adult plants assessed from yield trials, respectively.

A decrease in the concentration of Chl *a* and *b*, *Car*, assimilation (A) and tanspiration (*E*) rate in parental lines and among the population of 138 RILs examined in the LNK in comparison with the HNK is a typical consequence of physiological and biochemical transformations. It results from rearrangements of plant metabolism in response to occurring nutrient stress. The results are in accordance with the results presented by Kumar-Terawi et al. (2004) and Huang et al. (2004), who demonstrated that deficiency of macroelements caused an increase in the activity of antioxidant enzymes in maize and rice plants. Deficiencies of P, K and Ca in a medium resulted in increased activity of CAT, while N deficiency caused a not significant decrease in the enzyme activity. No significant differences were observed in the activity of POX between control plants and plants growing in LNK, although the means were higher for plants under stress. A deficiency in all the examined macro-elements resulted in an increase in SOD. Kumar-Tewari et al. (2007) demonstrated that N and K deficiencies modify antioxidative responses of the examined plants in different ways. The activity of CAT, POX and SOD in the population of RILs was different in comparison with parental lines. On the one hand, despite the different responses to stress caused by N and K deficiencies noted in parental lines, generally in the population of RILs nutrient stress caused a reduction in the activity of CAT and POX and an increase in the activity of SOD. Significantly different responses were often observed in selected RILs as a higher activity of CAT, POX and SOD. It should be emphasized that high SOD activity, lower CAT and the lowest for POX were found in the distinguished extreme tolerant group, and low activities of CAT and SOD and higher activity of POX were found in the susceptible group. The increase in SOD activity in the group of tolerant RILs in comparison to susceptible RILs may demonstrate different genetic background determining the response of RILs to abiotic stress (Kumar-Tewari et al. 2004, Shin et al. 2005).

Ward's agglomerative method was used to group RILs according to the d_CL, d_RN and d_LRL values, in order to reveal the effective response of each RIL to N-K stress. RILs were clustered in seven discrete groups (‘a’-‘g’), among which those differing extremely were clustered in groups ‘a’ and ‘g’. Characterization of individual clusters is presented in Figure 3. It was demonstrated that RILs responding to stress in a similar way as those clustered in groups ‘a’ and ‘g’ were identified in the neighboring clads (Figure 2). If we adopt biometric seedling parameters as a criterion for the response of RILs to nutrient stress, it may be concluded that Ward's agglomerative method can be used to

distinguish groups of RILs with extremely different responses from the examined population. The division has been verified with the use of the *k*-means method (data not shown). The examined population was divided into seven groups prior to its *de novo* agglomeration. It was demonstrated that the division was feasible. Variances between clusters were definitely higher than within groups. The d_LRL trait was the highest, highly significant statistic, which confirmed its usefulness as a predictor of the division of the population of RILs.

**Table 5. Variables in the model and standardized canonical discriminant function coefficients.**

| Variable | Wilk's λ | Partial λ | *F*-remove | p-level | Tolerance | 1-Tolerance | Root 1 | Root 2 |
|---|---|---|---|---|---|---|---|---|
| d_LRL | 0.642 | 0.333 | 128.020 | 0.0000 | 0.870 | 0.130 | 1.004 | -0.095 |
| d_CAT | 0.229 | 0.932 | 4.650 | 0.0112 | 0.953 | 0.047 | -0.012 | 0.782 |
| d_POX | 0.221 | 0.968 | 2.118 | 0.1245 | 0.929 | 0.071 | 0.157 | 0.368 |
| d_Car | 0.228 | 0.937 | 4.322 | 0.0153 | 0.165 | 0.835 | -0.624 | -0.870 |
| d_Chl *b* | 0.217 | 0.985 | 0.953 | 0.3881 | 0.176 | 0.824 | 0.316 | 0.263 |
| d_SOD | 0.218 | 0.979 | 1.378 | 0.2557 | 0.933 | 0.067 | 0.173 | 0.007 |
| d_A | 0.218 | 0.982 | 1.150 | 0.3198 | 0.697 | 0.303 | 0.135 | -0.315 |
| d_Chl *a* | 0.215 | 0.993 | 0.427 | 0.6532 | 0.169 | 0.831 | 0.084 | 0.541 |
| d_*E* | 0.215 | 0.994 | 0.370 | 0.6912 | 0.664 | 0.336 | 0.017 | 0.270 |
| Wilk's λ = 0.21388 F = 16.53 p < 0.0000 | | | | | | Eigenvalue | 3.133 | 0.131 |
| | | | | | Cumulative Proportion | | 0.960 | 1.000 |

Manschadi et al. (2008) used Ward's agglomerative method to group wheat cultivars with different tolerances to drought in terms of two traits (variables): growth angle and seminal root number. The authors demonstrated that seminal root architecture is closely linked to the angle of seminal root axes at the seedling stage and can be used in breeding for selection for water-limited environments.

Discriminant function analysis was employed by Ebdon et al. (1998) in the identification of low- and high-water use Kentucky bluegrass cultivars, while Kanbar et al. (2010) used it for selection of deep- and shallow-rooted plants in an early segregating generation of rice to identify drought-tolerant lines. In this study the greatest observable contribution comes from LRL (λ = 0.333, *F* = 128.020), and also from CAT, SOD; in addition Car turned out to be significant in the model. The result of canonical analysis was in agreement with the result of discriminant function analysis. RILs with high d_LRL 'in minus' were linked with deeper roots and, in the opinion of many authors, with better NUE (Rzepka-Plevneš et al. 1997a; Rzepka-Plevneš et al. 1997b; Lynch, 2007; Liu et al. 2008; Zhang et al. 2010).

In conclusion, - the range of morphological, physiological and biochemical changes described for the seedlings of recombinant inbred lines obtained from mature embryos was typical of such a study type. In spite of the many limitations and imperfections presented by Hazarika (2006), this fact according to the authors may support the commonly held opinion on the usefulness of the employed method as one of the possible laboratory methods for describing potential of plant response *per se* to nutrient stress at the seedling stage. Genotypes responding in different way to nutrient stress enable identification of molecular markers useful for selection in low-input breeding, such as could constitute interesting material for the monitoring of gene expression.

## ACKNOWLEDGMENTS

The author wishes to thank J. Kurek for help and efficient assistance during the experiments.

**Financial support:** This work was supported by the Polish Ministry of Science and Higher Education under grant, no. N N302 281936.

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**How to reference this article:**

SMOLIK, M. (X). (2013). Discrimination of population of recombinant inbred lines of rye (*Secale cereale* L.) for different responses to nitrogen-potassium stress assessed at the seedling stage under in vitro conditions. *Electronic Journal of Biotechnology*, vol. 16, no. 1. http://dx.doi.org/10.2225/vol16-issue1-fulltext-5