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Danuta Rzepka-Plevneš, Miłosz Smolik

Department of Horticultural Plant Breeding University of Agriculture in Szczecin,
8, Janosika Str., 71–424 Szczecin, Poland

POLYMORPHISM OF THE STORAGE PROTEINS IN RYE CULTIVARS AND POPULATIONS SELECTED FOR TOLERANCE TO NUTRIENT DEFICIENCY

ABSTRACT

Ten open–pollinated cultivars, two strains of rye and S_1, S_2, S_3 progenies obtained after selection directed towards tolerance to nutrient deficit were used in the study. The aim of the study was to compare the genotypes of the above mentioned rye populations by electrophoretically characterised of their secalin patterns.

The result showed that most of the tested rye cultivars revealed simi1ar but not identica1 elec–trophoretic patterns. Specific secalin patterns were obtained from all the cultivars, except cv. Arant and strain SMH 92. Differences in comparison to initial genotypes were observed in some rye popu–lations in S_1 and S_2 generations, including additional polypeptide of molecular weight 43 kDa. It was not linked with rye tolerance to nitrogen and potassium deficit in medium.

Key words: differences, electrophoresis, *in vitro* cultures, nutrient deficit, rye, storage protein, tol–erance,

INTRODUCTION

Improving the nutrient status of plants is of great value for food quality and to the health of both plants and humans (Horst, 2001). There are many examples showing that the plants with optimum nu–trient status are better adapted to the biotic and abiotic stress factors. Acording to Cakmak, (2001) "the plant nutrition studies will play a de–cisive role in establishing efficient and ecologically based nutrient management systems".

In literature of this subject there are many reports concerning the improvement of nutrient deficit tolerance of plants, especially of cereal cultivars (Górny, 1992; 1995; Hartmann *et al.* 1996). According to Stival da Silva *et al.* (2001) one of the ways to improve tolerance of plants to nutrient deficit is conventional breeding. The experiment re–ported here represents a contribution to this approach. It concerns ge–

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netic differences among ten rye cultivars, two breeding strains and their S_1, S_2, S_3 progenies selected towards tolerance to nitrogen and potassium deficit in medium under *in vitro* conditions. The effect of selection in successive generations and phenotypic variation among them was described earlier by Rzepka–Plevneš, (1999). In this paper the seed protein electrophoretic data concerning the genetic variability or similarity of above mentioned rye populations are presented. SDS–PAGE electrophoresis was used in the experiment. This method as very successful to identify genotypes of many plant species was reported by Laemmli, (1970); Shewry *et al.* (1985); Escribano *et a1.* (1998); Radić–Mieble *et al.* (1998); Hegde and Singhal, (2000); Zimniak–Przybylska *et al.* (2001).

The aim of the study was to compare the genotypes of nine open–pollinated cultivars, two strains of rye and S_l, S_2, S_3 progenies obtained from them after selection directed towards tolerance to nutrient deficit in medium by electrophoretically characterised of their secalin patterns.

MATERIAL AND METHODS

The study was conducted in 1998 – 2000. Investigations included two types of material: rye population cultivars and strains (Dańkowskie Selekcyjne, Dańkowskie Nowe, Dańkowskie Złote, Motto, Warko, Amilo, Pastar, Smolickie, Arant, Wibro, SMH 389, SMH 92) and genotypes S_1, S_2, S_3 derived from cultivars by three cycles of selection towards tolerance to N and K deficit in medium and sib–crossing of selected plants. Tolerance to N and K deficiency of the mentioned rye populations was examined in embryo cultures. The testing procedure was described earlier in the paper of Rzepka–Plevneš (1999).

The seed storage protein composition of the above mentioned material (original cultivars and lines – S_0 and selected populations S_1–S_3) and variation range of the protein level between them were analysed using SDS–PAGE gel electrophoresis technique. Protein extract was separated from the bulk samples consisting of 50 seeds for each tested population in three replications and from 30 individual seeds of S_0 and S_1 populations chosen after the experiment from: Dańkowskie Złote, Arant, SMH 389. Electrophoresis on 29: 1 polyacrylamide gels (4% – stacking gel and 12.5% – separating gel) and Tris–HCl–SDS buffer (pH 6.8 – stacking gel and pH 8.8 – separating gel) was run at a constant current 50 mA in two kinds of apparatus: Mini Protean vertical system (Bio Rad) and Multiphor II – horizontal system (Pharmacia LKB.). Gels were stained with Coomassie Blue R – 250. The proteins of each tested population were analysed qualitatively and quantitatively by computer programme for the analysis of polymorphic systems "Diversity one 1.3" (Pharmacia LKB). Genetic similarity was calculated by the analysis of phylogenetic tree constructed on the basis of

Jaccard's coefficient. Relative molecular masses (M_rS) of secalin polypeptides were estimated using the following standard protein: phosphorylase B (M_r 97.4 kDa), bovine albumin (M_r 66.0 kDa), actin (M_r 45 kDa), anhydrase carbonate (M_r 29.0 kDa), trypsin inhibitor (M_r 20.1 kDa) and α–lactalbumin (M_r 14.2 kDa) from Epicentre Technol–ogies, USA.

RESULTS

The studied material – rye cultivars and breeding lines revealed similar but not identical SDS–PAGE patterns of seed protein. Their polymorphism was observed, first of all, in the molecular mass of the identified on the electrophoregrams polypeptide (Table 1). The differ–ences in their tota1 number were not great, whereas molecular mass of the polypeptides ranged from 5.0 to 104.8 kDa.

HMW secalin fraction of rye cultivars and lines was the least numer–ous with respect to protein bands. In all the forms, two bands of 104.8 and 101.7 kDa were identified in this fraction. In the next fraction – ω four po1ypeptides were present. Their molecular mass ranged from 75.7 to 95.0 kDa. In particular rye genotypes they formed distinctive patterns consisting of two or three bands. There were also specific pat–terns formed by 4 to 5 protein bands ranging from 40.5 to 63.0 kDa within γ_{75} fraction. The most numerous fraction γ_{40} contained 9 – 11 protein bands with molecular weight of 5.0 to 34.0 kDa (Fig.1). The secalins of this fraction formed specific protein patterns differentiating rye cultivars under study. The cultivars Warko, Amilo, Arant, Pastar and both the breeding strains SMH 92 and SMH 389 were the excep–tions and had the same secalin pattern with bands of 34.0, 30.0, 20.5, 18.0, 16.5, 15.0, 11.9, 7.5, 6.1 and 5.0 kDa molecular mass.

The analysis of the phylogenetic tree of rye cultivars and lines indi–cated the great genetic similarity, ranging from 92.3 to 100% (Fig. 2). The greatest genetic similarity was observed between genotypes of cv. Arant and SMH 92 strain (group b) and Dańkowskie Złote and Motto (group a), the smallest – between cvs. Warko and Amilo.

Comparing SDS–PAGE patterns of secalins of S_1, S_2 and S_3 popula–tions with parental cultivars used for selection, it appeared that they did not differ from parental genotypes in the number of bands in HMW protein fraction (Table 1). There was no difference in the number of polypeptides between cultivars and their progeny, also in the next ω fraction in the case of cvs. Dańkowskie Złote, Dańkowskie Nowe, Warko, Amilo, Arant, Pastar and Dańkowskie Selekcyjne. In the re–maining genotypes of the mentioned fraction the observed differences concerned an additional polypeptide in S_1 or S_2 or both the generations. However, the total number of identified polypeptides in S_3 population was the same as in initial genotypes S_0 (Fig. 3).

Table 1

Protein band number in separated secalin fractions of rye cultivars and S_1, S_2 and S_3 populations obtained as result of selection for rye tolerance to N and K deficiency in medium under laboratory conditions.

Genotype	Secalins fraction																Total band number			
	HMW > 100				ω (100 kDa – 75 kDa)				γ_{75} (75 kDa – 40 kDa)				γ_{740} (< 40 kDa)							
	S_0	S_1	S_2	S_3	S_0	S_1	S_2	S_3	S_0	S_1	S_2	S_3	S_0	S_1	S_2	S_3	S_0	S_1	S_2	S_3
Dańkowskie Złote	2	2	2	2	2	2	2	2	5	5	6	6	11	11	11	11	20	20	21	21
Dańkowskie Nowe	2	2	2	2	2	2	2	2	5	6	6	6	11	9	10	11	20	19	20	21
Warko	2	2	2	2	3	3	3	3	6	5	6	7	10	10	9	10	21	20	21	22
Amilo	2	2	2	2	3	3	3	3	3	4	5	6	10	11	9	10	18	20	19	21
Motto	2	2	2	2	3	2	3	3	5	5	6	6	11	10	10	11	21	19	21	22
Wibro	2	2	2	2	3	2	3	3	5	5	6	6	9	9	9	9	19	18	20	20
Arant	2	2	2	2	2	2	2	2	5	4	5	5	10	10	10	10	19	18	19	19
Smolickie	2	2	2	2	2	3	3	2	4	4	4	5	11	9	9	11	19	18	18	20
Pastar	2	2	2	2	3	3	3	3	4	5	5	5	10	10	10	10	19	20	20	20
Dańkowskie Selekcyjne	2	2	2	2	3	3	3	3	5	6	6	6	11	11	10	11	21	22	21	22
Ród SMH 92	2	2	2	2	2	3	2	2	5	5	6	6	10	10	11	10	19	20	21	20
Ród SMH 389	2	2	2	2	2	3	3	2	4	5	5	5	10	8	9	10	18	18	19	19

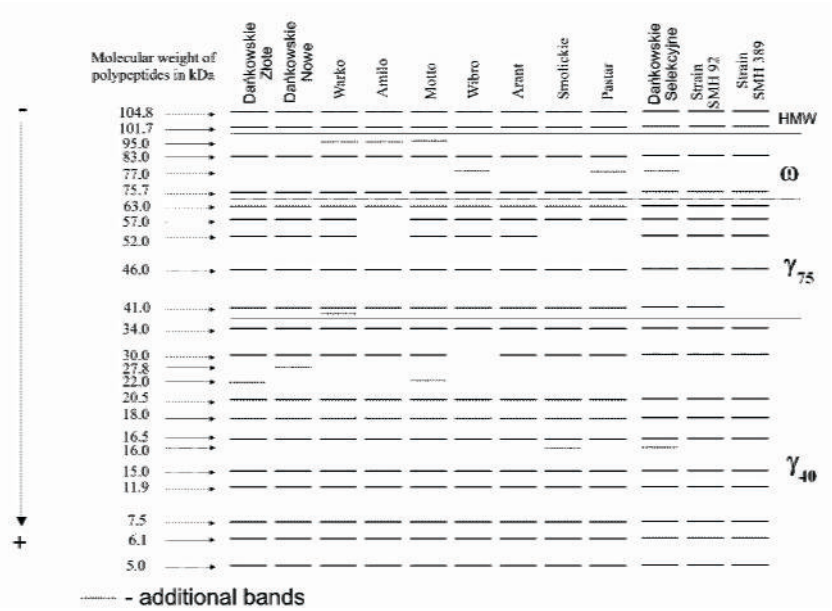

Fig. 1 SDS–PAGE secalin patterns of rye cultivars and breeding strains with specific bands differentiating the tested genotypes

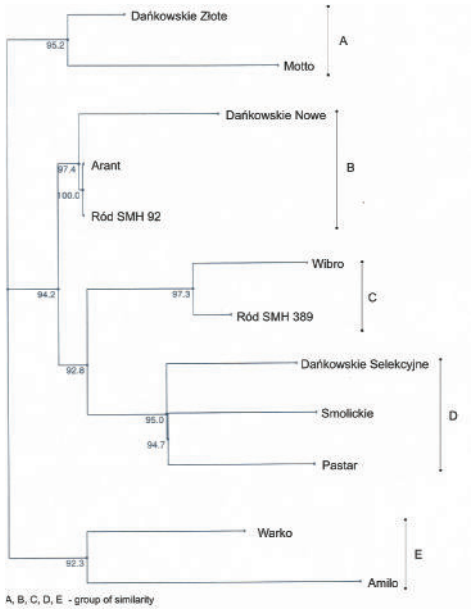

Fig. 2 Phylogenetic tree of genetic similarity of tested rye cultivars and breeding strains

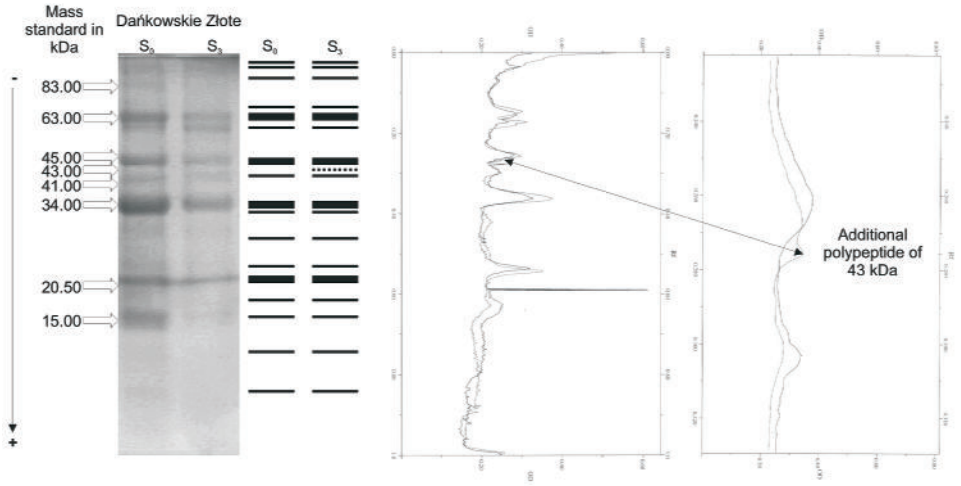

Fig. 3 SDS–PAGE secalins patterns obtained from storage peoteins isolated from bulk samples of grains of two rye genotypes Dańkowskie Złote before (S_0) and after (S_3) selection for tolerance to nutrition deficiency in medium. Densitometric analysis of an additional polypeptide of 43 kDa molecular weight in S_3 population of rye

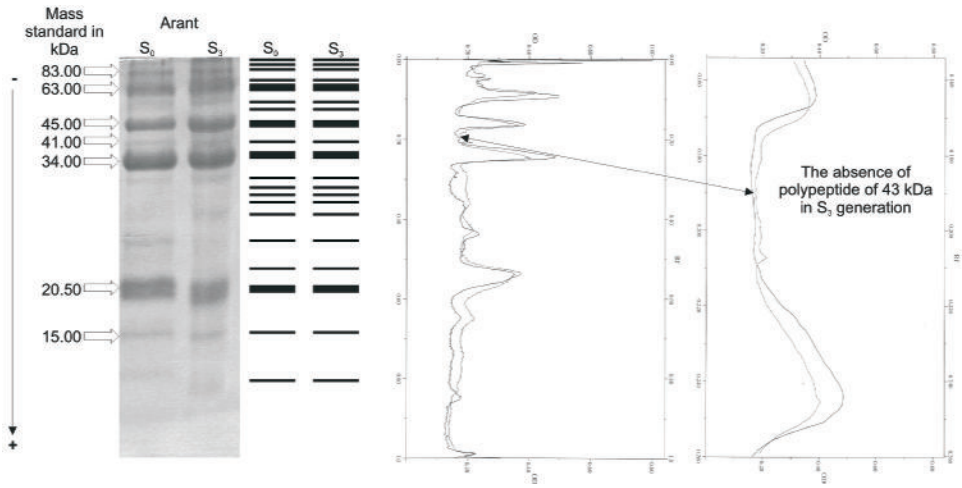

Fig. 4 SDS–PAGE secalins patterns obtained from storage peoteins isolated from bulk samples of grains of two rye genotypes Arant before (S_0) and after (S_3) selection for tolerance to nutrition deficiency in medium. Densitometric analysis of an additional polypeptide of 43 kDa molecular weight in S_3 population of rye

The protein fraction γ_{40} was characterized by slight changes in the number of observed polypeptides. No changes were noticed in the genotypes selected from cvs. Dańkowskie Złote, Smolickie and SMH 389 strain. The changes in other genotypes fluctuated, and S_3 did not differ in the polypeptide number from the initial generation S_0 (Table 1).

Differences in the structure of storage proteins between the tested rye populations were observed in the fraction γ_{75}. In this fraction, in some rye genotypes even in S_1 and in others in S_2 appeared on the electrophoregrams an additional, absent in S_0, band of 43 kDa, also present in S_3. This information was confirmed by the densitometric analysis of the patterns obtained for secalins of two generations – parental S_0 and selected S_3. It revealed the presence of the signals at the point OD in S_3 generation corresponding to the band of 43 kDa in all the rye genotypes, except genotypes selected from cv. Arant (Fig. 4) and SMH 389 strain.

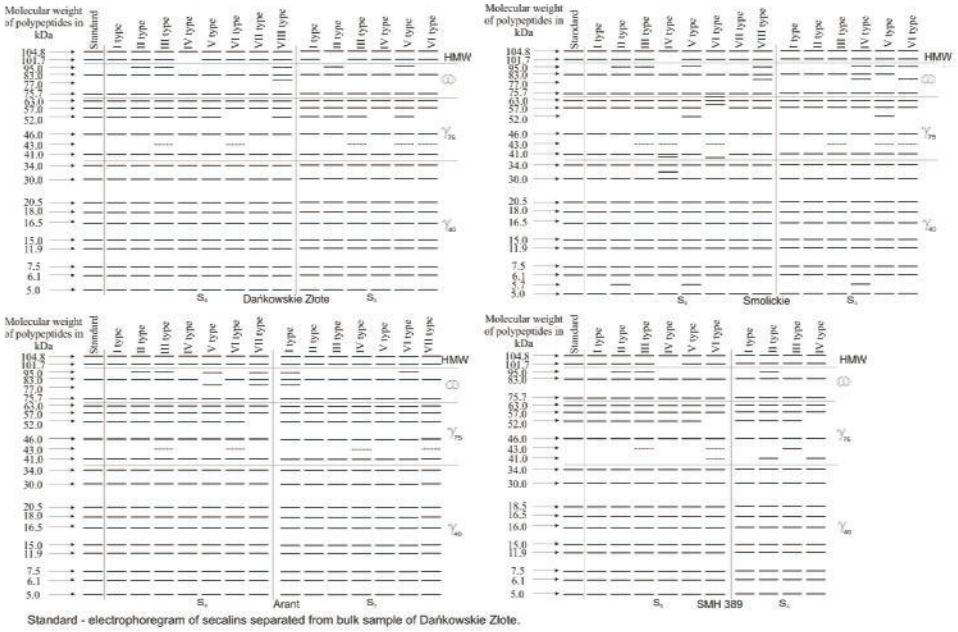

Fig. 5 SDS–PAGE secalin patterns of S_0 and S_3 Dańkowskie Złote genotypes obtained by electrophoresis of storage proteins isolated from individual grains of rye

The presence of the polypeptide of 43 kDa in γ_{75} secalin fraction of some rye genotypes was the reason for conducting additional studies on the individual grains of cvs. Dańkowskie Złote and Smolickie with that characteristic band and cv. Arant and SMH 389 – without it, and selected from them population S_3. SDS–PAGE patterns obtained from secalin analyses on bulk samples, were the standards for each rye genotype. Results show

that electrophoretic patterns of individual grains differed. There were 8 types of SDS–PAGE patterns in S_0 of cv. Dańkowskie Złote and Smolickie (Fig. 5). In Dańkowskie Złote the banding pattern type V dominated and VII in cv. Smolickie. In both the cases the patterns were characteristic of standard. In Dańkowskie Złote (S_0) the polypeptide of 43 kDa was present in patterns of type II and VI, in Smolickie (S_0) in 3 out of 30 analysed patterns. Populations S_3 selected from cvs. Dańkowskie Złote and Smolickie were characterised by similar to S_0 variation range and the polypeptide of 43 kDa in S_3 of these cultivars was observed in 8 and 9 patterns, respectively.

Evaluating the polymorphism of storage proteins of each of 30 individual grains of SMH 389 strain and cv. Arant it was found that there were 6 and 7 electrophoretic types within them (Fig. 5). In the case of SMH 389 strain the identical pattern to the standard was characteristic of the secalins of 12 grains and 11 grains in the case of cv. Arant. In both the mentioned genotypes the electrophoretic patterns with polypeptide of 43 kDa were obtained from 2 grains of each rye population. Variation range of S_3 population, of SMH 389 and cv. Arant was similar to S_0. In S_3 population, selected from SMH 389 strain, the band of 43 kDa was detected in 4 out of 30 electrophoretic patterns (types III and IV) whereas in S_3 derived from Arant only on 2 electrophoregrams (types IV and VII).

DISCUSSION

The response of barley, oats and rye seed to the nutrient deficit stress has been described by Górny, (1992; 1995), Hartmann *et al.* (1996), Rzepka–Plevneš, Tomczak, (1996) and Rzepka–Plevneš, (1999). Results from all these experiments conducted in the field, hydroponic culture and embryo *in vitro* culture suggested that cultivated crops are characterised by high variability of tolerance to nutrient deficiency. For example, in rye cultivars the frequency of tolerance seedlings varied from 44 to 99%. The growth criteria (seedlings height, roots length) were significantly affected by nutrient deficit. Our results demonstrated, that both species (oats and rye) reacted similarly to the decreased level of nutrient components in the medium. They showed the differentiation of morphological traits: the height of seedlings and length of roots. It was found that seminal root system could constitute significant selection criterion in rye.

However, no significant differences were observed between rye cultivars at the genotype level. Electrophoresis data presented in this paper showed that genetic similarity between them ranged from 92.3 to 100%. Our present results correspond to the previous data obtained by Niedzielski *et al.* (1997).

Selection for rye tolerance to nutrition deficiency under *in vitro* culture increased differences in tested traits (seedlings height and roots length) between parental cultivars and their progenies. In S_1 generation

a decrease in the frequency of the seedling with long seminal roots was revealed, in S_2 – the frequency of these seedlings were similar to in parental generation and in S_3 significantly increased (Rzepka–Plevneš, 1999).

The result of the present investigations confirmed that S_1, S_2 and S_3 populations differ from parental genotypes in the structure of seed storage proteins. Polymorphism concerning molecular mass was observed in SDS–PAGE patterns of polypeptides. These differences depended on the properties of the examined genotypes and were most frequently visible in the fractions of low–molecular–mass proteins γ_{75} and γ_{40}. In γ_{75} fraction of the majority of S_2 and S_3 genotypes an additional, polypeptide of 43 kDa has been identified. Its presence was confirmed by densitometric analysis of electrophoretic patterns. The only exceptions were two rye genotypes selected from cv. Arant and SMH 389 strain in which the polypeptide of 43 kDa was not found on either of the obtained seed protein patterns. Electrophoretic analyses of secalins isolated from individual grains of some rye genotypes demonstrated its presence in all rye populations but in some of them the frequency of these genotypes was very small and the mentioned polypeptide was, for example, present in 3 out of 30 analysed SDS–PAGE patterns.

In many author's opinion (Carillo *et al.* 1990; Waga and Grzywa, 1995; Waga, 1996; 1997; 2000; Waga and Węgrzyn, 2000) the storage proteins of cereals may be used for the search of genetic markers linked with important quality characters of crops. For example, gene *Hor–2*, responsible for the polypeptides of B group barley cultivars, is coupled with a recessive gene *Mla* determining barley resistance to powdery mildew *(Erysiphe graminis* f. sp. *hordei).* The identification of this coupling was used as a genetic marker in selection for the resistance to this pathogen (Timothy *et al.* 1983). The studies of Waga and Grzywa, (1995) are another example. These authors proved that the resistance of barley to powdery mildew, controlled by *Mla* gene is closely connected with the presence of polypeptides migrating quickly on the gel. In Waga and Węgrzyn's (2000) opinion electrophoretic pattern of wheat gliadins and glutenins may be used as a selection criterion for technological value of wheat. They also link different gliadin groups with wheat productivity and many other resistance features. Apart from that, they suggest the possibility of using storage proteins as molecular markers of the traits with a complicated genetic system.

In our experiment material for the study ($S_1 – S_3$) originated from the selection for high tolerance to nutrition deficiency, not from crosses. Therefore we managed to find the differences between successive selected generations in the frequency of tolerant seedlings, their morphological traits and seed protein polymorphism. Further studies focus on the analysis of the relationship between tolerance to nutrient deficit and seed protein, and also DNA electrophoretic patterns in F_2 progenies of crosses between tolerant and non–tolerant parents.

CONCLUSION

The results of the conducted experiments show that, there is 92.3 to 100% similarity between Polish rye cultivars. Differences in the structure of seed protein between them are observed in the molecular mass of polypeptides. The greatest polymorphism of seed protein is observed in the fraction γ_{75}.

The experimental material for the study (S_1, S_2, S_3) originated from the selection directed towards high rye tolerance to nitrogen and potassium deficiency and sib–mating plants. It was characterised by greater genetic differences in respect to parental cultivars. These differences were observed especially in the γ_{75} fraction of seed proteins. Additional polypeptide of 43 kDa in this fraction present in S_3 and in some populations in S_1 and S_2 generation was not linked with rye tolerance to nitrogen and potassium deficiency.

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