ABSTRACT: *Bromus secalinus* L. is an annual speirochoric species growing in agroecoses of winter crops. In the past, *B. secalinus* L. could be found throughout Poland. The regression of the species has caused it to be classified as a rare species threatened with extinction. The study was conducted in the 2007–2010 time period, in north-eastern Poland where a considerable increase in the number of sites and abundance of *B. secalinus* were observed.

The plant material was collected from agroecoses of winter crops, at 14 sites representing the habitat diversity of the species occurrence. Eight morphological features were taken into account when evaluating populations of *B. secalinus*. The morphological diversity of the populations of *B. secalinus* was correlated with different habitat conditions (type, pH of soil and cultivated plant). The populations growing on the sites with optimum trophic and moisture conditions as well as those populations growing in very poor conditions were found to differ the most. A comparison of *B. secalinus* was done and six groups of cluster similarities were found using the agglomeration method. Electrophoretic analysis of seed storage proteins of *B. secalinus* L from the studied populations were performed using SDS-PAGE. Analysis revealed relatively high genetic polymorphism irrespective of whether genetic variation was compared between the populations from different sites or between the subpopulations from only site number 4. Three homologenous groups were formed which were related to the cultivated plant coverage, and partially, to the type of soil. In the investigated populations and subpopulations, protein bands of molecular weight from 8 to 68 kDa were observed. The SDS-PAGE method was found suitable for the assessment of genetic variation in populations of *B. secalinus* L from many sites as well as within the subpopulations from one site.

KEY WORDS: *Bromus secalinus*, morphological features, polymorphism, storage proteins, variability

1. INTRODUCTION

Segetal vegetation undergoes continuous transformations. Certain specialised elements of agroecoses disappear mainly as a consequence of extensive cultivation. Speirochoric species and the species of a narrow ecological amplitude are particularly sensitive to anthropopressure (Korniak 1992, Andreason et al. 1996, Hocžół 1998, Korniak and Holdynski 2006, Kropáč 2006, Lososova and Simonova 2008). *Bromus secalinus* L. is an archaeophyte, classified as no-homeland species (Thellung 1925) and is included in the group of archiophyta anthropogena (Zając 1979, Mirek 1981). *Bromus secali-
**nus** L. is a stable hybrid of *B. commutatus* and *B. racemosus* (Scholz 1970). *B. secalinus* L. is evolutionarily adapted to spread with the seeds of cereals and considered to be an obligatory (Zająć 1979) or facultative speirochor (Kornaś 1987). The species has been included in the group of endangered species, characterized by significant fluctuations of occurrence frequency in many regions of the country and the world. Recently, both in Poland and in Europe, it has been classified as a threatened species (Warchołińska 1994, Żukowski and Jackowiak 1995, Anioł-Kwiatkowska 1998, Magločky 1999, Preston et al. 2002, Nowak et al. 2003, Jeziorska-Domaradzka and Kuźniowski 2006), yet at the same time, it is considered a weed whose population grows significantly along with the number of sites of its appearance. This later tendency is clearly observed in the area of eastern Poland (Skrajna et al. 2005, Kapeluszny and Haliniarz 2007, Korniak and Dynowski 2009, Rzymowska et al. 2010). The increased frequency of *B. secalinus* has also been reported e.g. in Germany (Moray et al. 2003, Moray 2005), in Canada and the southern states of the USA (Stone et al. 2006), and in Romania and Turkey (Moray 2005).

The lack of diversity within a species leads to a narrowing of the genetic pool. In turn, a decrease is created in the number of localities and in the population abundance. The threatened and expansive species are subjected to monitoring. The population variation is also studied by morphological observations (Skrajna and Skrzyczyńska 2007) or with the help of protein markers (Kubicka et al. 2005) or molecular markers (Jin and Li 2007).

One of the cheapest and most commonly used methods in taxonomy and phylogeny investigations of the Poaceae flora species is SDS-PAGE (Chen et al. 1997, Aiken et al. 1998, Oja et al. 2003, Kharazian 2008, Tamkoc and Arslan 2011). In cultivated plants this technique is mainly used for the determination of polymorphism of crop plants. This technique is particularly used for the identification of genotypes, variation of offspring, and offspring similarity to parent plants (Carillo et al. 1990, Rzepka-Plewniš and Smolik 2001, Rzepka-Plewniš et al. 2003, Kubicka et al. 2005).

In view of the dynamic changes in the occurrence of Bromus secalinus in north-eastern Poland, this study was undertaken to assess the phenotypic variation and polymorphism of seed storage proteins in selected populations of this species.

### 2. THE STUDY AREA

The study area was the north-eastern region of Poland (Fig. 1). Small traditionally-run farms are dominant in this area and many of the farmers are poor. Gross negligence in agrotechnology has resulted in considerable contributions of weeds as well as the presence of various agricultural pests.

<table>
<thead>
<tr>
<th>No</th>
<th>Site (local name)</th>
<th>Mezoregions (Kondracki 2002)</th>
<th>Co-ordinates</th>
<th>ATPOL Raster 5 km</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bryżgieł</td>
<td>Eastern-Suwałki Lake district</td>
<td>53 59 54,1N; 23 03 42,1E</td>
<td>FB-1911</td>
</tr>
<tr>
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<td>Eastern-Suwałki Lake district</td>
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<td>FA-9811</td>
</tr>
<tr>
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<td>Eastern-Suwałki Lake district</td>
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<td>FB-1901</td>
</tr>
<tr>
<td>4</td>
<td>Horodnianka</td>
<td>Augustów Plain</td>
<td>53 39 18,9N; 23 06 41,3E</td>
<td>FB-1901</td>
</tr>
<tr>
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<td>Rzepiska</td>
<td>Bielsk Plain</td>
<td>52 49 59,0N; 23 33 31,0E</td>
<td>GC-4311</td>
</tr>
<tr>
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<td>Golaszyn</td>
<td>Łuków Plain</td>
<td>51 57 44,3N; 22 21 56,6E</td>
<td>FD-4610</td>
</tr>
<tr>
<td>7</td>
<td>Rudnik</td>
<td>Łuków ŁPlain</td>
<td>51 54 40,7N; 22 44 11,0E</td>
<td>FD-5801</td>
</tr>
<tr>
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<td>Popławy</td>
<td>Łuków Plain</td>
<td>51 59 33,5N; 22 30 51,9E</td>
<td>FD-4710</td>
</tr>
<tr>
<td>9</td>
<td>Niwiski</td>
<td>Siedlec Upland</td>
<td>52 13 14,9N; 22 09 57,4E</td>
<td>FD-2401</td>
</tr>
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<td>10</td>
<td>Bojmie</td>
<td>Węgrows Depression</td>
<td>52 12 13,8N; 21 57 15,4E</td>
<td>FD-2300</td>
</tr>
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<td>11</td>
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<td>Węgrows Depression</td>
<td>52 12 20,4N; 21 59 34,6E</td>
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<tr>
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<td>Groszki Star</td>
<td>Kaluszyn Upland</td>
<td>52 12 08,5N; 21 54 23,5E</td>
<td>FD-2201</td>
</tr>
<tr>
<td>13</td>
<td>Barczacja</td>
<td>Kaluszyn Upland</td>
<td>52 09 04,7N; 21 37 59,9E</td>
<td>FD-2110</td>
</tr>
<tr>
<td>14</td>
<td>Tyborów</td>
<td>Kaluszyn Upland</td>
<td>52 09 52,3N; 21 40 03,7E</td>
<td>FD-2110</td>
</tr>
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</table>
of floristically rich agrophytocenoses. The crops are mainly cereals and their yields are lower than the country’s average (Central Statistical Office (GUS) 2009).

Over this area, 14 sites with *Bromus secalinus* were chosen for the study. These sites represent the ecological amplitude of *B. secalinus* habitats and are located in the 7 mezoregions characterised in Table 1.

3. MATERIAL AND METHODS

Observations were carried out in the 2007–2010 time period. The study involved making phytosociological releves using the Braun-Blanquet method. The frequency and abundance of *Bromus secalinus* populations were defined. At the sites selected (Table 2), the soil unit was localised in the fields with winter crops and represented different habitat conditions. The soil unit was established on the basis of soil-agricultural maps (1:5000 scale). The surface layer soil pH was measured by the colorimetric method.

From each site, 30 specimens of *Bromus secalinus* were randomly collected (Malicki and Prędyś 1980) in the milk-wax maturity phase. Eight morphological features were analysed: the tuft height, the number of culms in the tuft, the height of generative shoots, the panicle length, the number of spikelets in the panicle, the number of kernels in the spikelet, the number of kernels in the panicle and the seed production.

Morphological data were subjected to statistical analysis with the use of the Statistica 6.0 packet. Distribution of the features studied did not conform with the normal distribution, so non-parametric tests were used. Statistical analysis was based on the Kruskal-Wallis test (for $P \leq 0.05$), which is a non-parametric alternative of ANOVA. Results are illustrated by box plots showing the median distribution and maxima and minima of the features. Spearman rank correlations between seed production and the other traits were checked for all sites).

The multidimensional cluster analysis was applied to compare the plants from particular populations. All of the above-mentioned traits were taken into consideration for the comparison. The analysis was made by the method of agglomeration of a single link using Euclidean distance. The traits were subjected to standardization depending on the type of statistical analysis performed.

Electrophoresis of storage proteins from the seeds of *B. secalinus*, was performed applying the modified SDS-PAGE method adapted to rye by Kubicka et al. (2005).

The seeds of *B. secalinus* were randomly collected from the populations growing at 14 selected sites (Table 1). Additionally, the genetic variation was assessed within two
subpopulations from distant mezoregions, sites 4 and 9 as well as with the different type of soil and cultivated plant coverage. For the measurements, four seeds of *B. secalinus* were chosen from each particular population in two repetitions. The seeds were ground with a metal spatula. Storage proteins were extracted from the seeds with a 300 ml buffer treatment (0.063 M Tris, 10% glycerol, 2% SDS, 5% marcaptoethanol bromophenol blue) for a 1.5 hour accidental vortexing of samples. The samples were denaturated for 10 minutes in a water bath at 95°C.

Electrophoresis was carried out using a vertical apparatus (Hoeffer). An aliquot of protein extract (40 ml) was resolved in stacking gel (T 4%) at 30 mA and in separating gel (T 10%) at 45 mA until tracking dye reached the bottom edge of the gel. Resolved protein bands were stained with 0.25% CBB R-250 solution in (10% of methanol and 40% acetic acid). The gels were dried and evaluated on Gel Doc 1000/2000 (Bio-RAD). To estimate population similarity as revealed by protein patterns, agglomerative hierarchical clustering (AHC) based on the Jaccard similarity coefficient were performed, using the program XLSTAT 2010.

### 4. RESULTS

#### 4.1. Occurrence of *Bromus secalinus*

*Bromus secalinus* is frequently present in north-eastern Poland. It is a popular weed among the winter crops and grows on soil of different types. The pH values of the soil on which it can grow range from slightly acidic (pH 5.9) to basic (pH 7.5) (Table 2). Analysis of the habitat conditions of this species occurrence has shown that its presence is correlated with pH range and certain compactness of soil.

The populations growing at the highest pH sites (sites 1, 2 and 7) reached a 20–25% degree of coverage. At the poorer site no. 11, the degree of coverage was 15%, but at the other sites it did not exceed 10%. *Bromus secalinus* is most often found in patches of *Aperion spica-venti* communities and the *Vicietum tetraspermae* group (Table 2).
4.2. Morphological variation

Statistical analysis was performed using the Kruskal-Wallis test (Table 3) and Spearman rank correlations (Table 4). The test results showed that morphological features of *Bromus secalinus* individuals growing in cereal canopy at the 14 sites studied were statistically significantly different (Fig. 2). These features were also correlated with the pH soil, soil unit type, and cultivated plants coverage (Table 4).

The tallest individuals were found on brown soil at sites 1 and 2. Brown soil had the highest pH (7 and 7.5). The average heights of the tallest individuals were slightly different: 137.6 and 140.7 cm (Tables 1 and 4). The smallest individuals grew at site 11, on a poor rye complex, where the soil pH was 6.8 and degree of coverage was 55% (Fig. 2A, Table 2). A similar relation was observed for the height of generative shoots (Fig. 2C). The smallest variation was noted in the number of culms in the tuft. The average varied between 1,9 and 5,1 (Fig. 2B). This feature was statistically significantly depending on the pH soil and the type of soil unit, but not on the degree of coverage (Table 4).

The panicle length showed a large variation (H = 119.40* P = 0.000) and the greatest correlation with the trophism of sites (Fig. 2D; Table 3). By far the greatest panicle length was noted for the population growing at site 2 (17.5 cm) where the pH was 7.0 in cereal-fodder strong complex and degree of coverage was 80% (Table 2). Panicles of similar lengths were found in the populations growing at sites 1, 3 and 14. The average height amounted to 16.1 cm, 15.1 cm and 17.7 cm, respectively. The plants from populations 1–3, 6–7, and 14 with compact soils having pH levels between 5.9 and 7.5, were characterised by a greater number of spikelets in the panicle, with the average varying from 19.4 to 21.3 (Fig. 2E).
Fig. 2. Ranges of variation of selected traits: A – tuft height, B – number of culms in the tuft, C – height of generative shoots, D – length of panicle, E – number of spikelets in the panicle, F – number of kernels in the spikelet, G – number of kernels in the panicle, H – seed production. 1–13 – study sites (see Table 1, 2).
Morphological features and polymorphism storage protein in *Bromus secalinus*

Such features as the number of kernels in the spikelet (Fig. 2F) and number of kernels in the panicle (Fig. 2G) were also determined by the habitat conditions, mainly by the pH of soil, as indicated by the highest average of these features for the populations growing at sites 1 and 2 (Table 2).

A large variation was observed in the seed production that was correlated with features characterising the inflorescence of particular populations, differed significantly between populations ($H = 121.57^*$; Fig. 2H, Tables 3 and 4).

Analysis of relations between pairs of traits has shown that the majority of them were positively correlated. The strongest correlation was found between the height of generative shoots and the length of the panicle (0.72), and the height of generative shoots and the number of spikelets in the panicle (0.63). As far as the inflorescence traits are concerned, the strongest correlations were established between the length of the panicle and the number of spikelets in the panicle (0.73), the length of panicle and the number of kernels in the panicle (0.70), as well as the number of kernels in the panicle and the number of spikelets in the panicle (0.88). The seed production was correlated the strongest with the number of culms in the tuft (0.74), the tuft height (0.57) and number of kernels in the panicle (0.56). No significant correlation was found between the number of culms in the tuft and the length of panicle, the number of spikelets in the panicle, or the number of kernels in the spikelet and panicle.

A comparison of *Bromus secalinus* morphotypes from all the studied sites (Table 1) is presented in a dendrogram (Fig. 3). The number of clusters was established on the basis of the agglomeration of a single bond and the Mojena rule. The clusters were arranged in 6 groups. The first group included the populations growing at sites 1, 2, 3 and 14. The highest similarity was between plants from sites 1 and 2. They were located close to Wigierski National Park and they formed a cluster in the first step. The plants from sites 14 and 3 joined this cluster only at the 8th step. Site 3 (Magdalenowo) is also located on the area of Wigierski Park, but site 14 (Tyborów) is located on the opposite side of the area of investigation. The second group of clusters included the plants from populations growing at sites 4, 12 and 13. Sites 12 (Groszki Stare) and 13 (Barcząca) are located in the vicinity of mezoregions (Węgrów Depression and Kaluszyn Upland) while site 4 (Horodnianka) is located in the distant North-East of Augustów Plain mezoregion. The plants from sites 4 and 12 included clusters in the second step while plants from site 13 included clusters in the 5th step. The next cluster was made of specimens growing at sites 8, 10 and 7. Sites

![Dendrogram](image_url)

**Fig. 3.** Dendrogram of similarities of *Bromus secalinus* morphotypes from north-eastern Poland, obtained by the method of agglomerations of the single bond using Euclidean distance.
Fig. 4. Diagram of protein bands among accessions of *Bromus secalinus*: A: populations from different sites 1–13 (see Table 1, 2); B: subpopulations from site 4; C: subpopulations from site 9. kDa – molecular weights (in kiloDaltons) of reference marker proteins.
Morphological features and polymorphism storage protein in *Bromus secalinus*

8 (Popławy) and 7 (Rudnik) are located on the Łuków Plain. Site 10 (Bojmie) is located on the area of the Węgrów Depression. The fourth group was made by the plants from the distant 5 and 9 sites (Bielsk Plain and Siedlce Upland), which joined the group at step 4. The last two groups included the plants from individual populations growing at sites 6 and 11. These two last groups were very different from the plants from the other sites.

4.3. Genetic variation

The plants of *Bromus secalinus* collected at different sites in north-eastern Poland showed considerable polymorphism of storage proteins. The highest number of bands corresponded to the proteins of molecular weight varying between 8 and 68 kDa, both in the populations as well as the subpopulations from sites 4 and 9 (Fig. 4A–C). Almost all *B. secalinus* populations (with the exception of 7) as well as the subpopulations 5–13 at site 4, contained storage proteins equal to the molecular weight of 68 kDa. Proteins of a molecular weight equal to 60 kDa were observed in six populations (3, 5, 6, 8, 9 and 13) of the *B. secalinus*, as well as the subpopulations 5–13 (site 4) and 5,7 (site 9). Most populations, with the exception of 7 and one subpopulation (site 9), contained protein of a molecular weight equal to 48 kDa. However, proteins of a weight equal to 40 kDa were observed in the following populations (1, 2, 4, 7, 10, 11, 12 and 14) as well as subpopulations (2–5, 7, 9 and 10) (site 9). In both populations (2–5, 7–8, 10 and 13) as well as subpopulations (1–5 and 11) from site 4, proteins of a molecular weight of 38 kDa were present. In some populations (5–6, 8–9 and 13), and almost in all subpopulations from site 4, low-molecular proteins of a mass equal to 10 kDa were observed (Fig. 4A–C). Protein bands characteristic for populations originating from 14 sites had a molecular weight of: 43 and 15 (Fig. 4A).

Similarly, in subpopulations *B. secalinus* at site 4 and 9, the appearance of protein of a molecular weight of 8 to 68 kDa was observed. Higher protein molecular weight ranging between 20 and 68 kDa, was observed in subpopulations *B. secalinus* from site 4. In the subpopulations from site 9, protein of a molecular weight below 34 kDa was dominant (Fig. 4B and 4C). Protein bands of a molecular weight of 62 kDa, 34 kDa and 25 kDa were observed in subpopulations from both sites (4 and 9). The protein which was only present in subpopulations from site 4 had the following molecular
weight: 45 kDa and 20 kDa. However, in site 9 only, the following molecular weights of protein from subpopulations 13 kDa, 12 kDa and 8 kDa were observed (Fig. 4B and 4C).

The genetic similarity between the Bromus secalinus populations ranged from 0.38 to 1.0 (Fig. 5). Three groups of populations as indicated by protein electrophoresis pattern Bromus secalinus were identified. The first group included the greatest number: there were 8 populations (1, 2, 4, 7, 10, 11, 12 and 14), including the populations from sites 4 and 10, which showed the greatest genetic similarity of 1.0, although they grew in distant mezoregions (Augustów Plain and Węgrów Depression). The second group included 5 populations (5, 6, 8, 9 and 13). The third group had only one population growing at site 3, which was the most genetically distinct from the other populations. Great genetic variation was noted between the B. secalinus subpopulations collected from site 4 of the Eastern-Suwałki Lake district (coefficient of similarity varied from 0.38 to 0.94). Within this population, three groups with genetic similarity were also identified. The first group included 3 subpopulations, the second – 9 subpopulations and the third – 2 subpopulations (Fig. 6).

The highest genetic similarity of 0.61 to 0.90, was found in the subpopulations growing at site 9, localised in the mezoregion of Siedlce Upland (Fig. 7). The protein profiles contained the highest number of bands; from 18 to 31, but the majority of them were monomorphic. Three groups of similarities were identified; the first included 4 subpopulations, the second 3 subpopulations, and the third, 5 subpopulations.

The genetic distance between the populations originating from different sites was slightly shorter than between the subpopulations from the same site no. 4. (0.38 to 0.94). This observation means that the populations of Bromus secalinus from the sites studied (except for sites 4 and 10) and the subpopulations growing at site 4 show considerable genetic differences which helps in the adaptation of this species to the environment. The populations and subpopulations contain different genotypes of B. secalinus. But the subpopulations growing at site 9 were found to show greater genetic similarity.

5. DISCUSSION

Some small fluctuations in the share of Bromus secalinus in agrocenoses of eastern

Fig. 6. Genetic distances among B. secalinus subpopulations from site 4 based on Jaccard similarity coefficient.
Morphological features and polymorphism storage protein in *Bromus secalinus*

Poland over the last 30 years has been noted. It is due to the traditional way of farming in this region, that it has not been necessary to include the species from this area the group of endangered plants, (Korniak 1992, Skrzyczyńska 1994, Skrzyczyńska and Skrajna, 1999, Skrzyczyńska and Rzymowska 2001, Rzymowska *et al.* 2006, Rzymowska and Skrajna 2011a). The dynamic increase of abundance of *Bromus secalinus* populations and density of stands in the studied area, is probably the result of using unqualified seed material, a high share of winter cereals in crop structure, simplification of crop rotations, and cultivation abandonment. All these factors also contribute to the frequent occurrence of other sperochoric species in this area, e.g., *Agrostemma githago* and *Avena strigosa* (Korniak 1997, Skrzyczyńska *et al.* 2005, Rzymowska and Skrajna 2011b). Similar reasons for the increase of the *B. secalinus* share in agroecosystems were suggested by Petersen (2006). He argued that expansion of *B. secalinus* was also affected by the use of herbicides from the group of ALS inhibitors (Atlantis, Atrybut). Studies carried out by Moray *et al.* 2003 indicate a close relationship between the number of *B. secalinus* specimens in cornfields, and such factors as cereal sowing date and soil cultivation methods.

According to our observations, this species is characterised by high plasticity with respect to habitat conditions. *B. secalinus* has been noted in the majority of soil types, except for extremely poor soils. Zarzycki *et al.* (2002) claim that *Bromus secalinus* prefers habitats with moderate trophic and moisture content. Cousens *et al.* (1988) and Moray (2005) reported small differences in the parameters of *Bromus sterilis* and *B. secalinus* growing at different localities in the same year, and their increased competitiveness due to favourable weather conditions.

The observed deviations from the median of the morphological traits of the populations from north-eastern Poland, were positively correlated with habitat conditions. The greatest differences were observed for the populations at sites 1, 2 and 11. In close crop fields, *Bromus secalinus* populations tillered weakly and most often created 1–2 growing stems over the crop field. The lowest individuals of specimens were found on the poorest soil type, e.g. at site 11, at which the coverage degree of the winter crop was the smallest. Similarly, individual specimens growing on idle land were low; their mean height was 90 cm.

Fig. 7. Genetic distances among *B. secalinus* subpopulations from site 9 based on Jaccard similarity coefficient.
(Majda et al. 2007). Differences in the height of the plants were determined by light accessibility, as compact crop means more shadow conditions and *B. secalinus* is a light-loving species (Zarzycki et al. 2002).

Results of our study have proved that one plant of *B. secalinus* is able to produce a large number of seeds. However, the seed production observed in this study at the majority of sites studied, was lower than that given by Moray et al. (2003) from Germany who observed 800–1000 seeds, or by Majda et al. (2007) from idle land in Lublin district who noted 1082 seeds. The seed production is not only a feature specific of a species but depends on many ecological factors (Holzner et al. 1982, Lutman 2002). Our results have confirmed this relation. The individuals producing the largest number of seeds were growing at site 2 offering the best conditions for development. At this site, 75% of the population produced over 1200 kernels per individual plant.

The *Bromus secalinus* populations studied were characterised by greater phenotypic variation than genetic variation of the seed storage proteins. This variation was true despite a rather low genetic similarity between populations, except for sites 4 and 10. Higher genetic polymorphism was noted between the subpopulations growing at site 4, where the cultivated plant was rye growing on poor soil. This range of variation is typical of other allogamous species with widespread occurrence e.g. *Secale cereale* (Kubicka et al. 2005) or *Elytrigia repens* (Prokudin, 1982). Greater genetic variation was often observed for inbred lines of rye selected from the same varieties than in lines from different initial materials (Kubicka et al. 2005). This observation was confirmed by our results, evidencing a greater genetic distance between the subpopulations growing at site 4, which was probably connected with the cultivated plant. Unqualified seed material of rye used for sowing was polluted by *B. secalinus* which are similar to poorly developed seeds of rye and are difficult to get rid of during purification. Higher genetic variability was a consequence of the appearance of new genotypes of *Bromus secalinus* because of the use of polluted sowing material coming from different sources. The greater similarity between the subpopulations growing at site 9 was noted. This similarity was because triticale the coverage plant, was cultivated on better soil. The qualified seeds probably were used in sowing.

The SDS-PAGE study of storage proteins extracted from kernels originating from *Bromus secalinus* populations growing in north-eastern Poland, has proved their statistically significant polymorphism. This polymorphism is particularly pronounced for proteins with a molecular weight between 8 and 68 kDa.

The results are consistent with those reported by Kharazian (2008), who observed a similar range of differentiation in the molecular weight of storage protein (35–76 kDa) in the diploid seeds of the *Aegilops* species. However, Tamkoc and Arslan (2011) by comparing three *Poa* diploid species, obtained higher variability on the storage protein level which was equal to 10–128 kDa.

Some protein bands present in the *B. secalinus* population originating from different sites were observed in the subpopulations growing at sites 4 and 9. Two protein bands were characteristic for the investigated *B. secalinus* population. There was similarity in the subpopulations from sites 4 and 9 which also contained several characteristic storage proteins. Some of the identified storage proteins were also observed by Chen et al. (1997) in three *Bromus* species, namely: *inermis*, *sterilis* and *tectorum*. These were proteins of a molecular weight above 45 kDa. They also showed a similarity on the storage protein level between *Poaideae* and *Bromeae* genera and *Triticeae* tribes. Storage proteins weighing 38 kDa were identified in populations and subpopulations at site 4. These results were also observed by Chen et al. (1997) but in *Panicoidaeae* species genera.

The above presented and discussed results show that the SDS-PAGE method of electrophoresis of storage proteins is well suited for assessment of genetic polymorphism of cultivated crops. The SDS-PAGE method is also suitable for the investigation of the genetic variation of *Bromus secalinus* population and subpopulations growing in north-eastern Poland.

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