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CHARACTERIZATION OF *Aegilops kotschyi* Boiss. x *Triticum aestivum* L. HYBRID LINES

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Abstract

A study of four F_5 and one BC_1F_1 *Aegilops kotschyi* Boiss. x *Triticum aestivum* L. hybrid lines was conducted to determine their quantitative morphological and qualitative features as well as a molecular investigation was carried out. Observations of ten quantitative traits showed that the F_5 hybrid lines exhibited intermediate values between *Ae. kotschyi* Boiss. and *T. aestivum* L., or had similar traits to one of the parents. These hybrid lines had a significantly lower number and weight of grains per main spike, main spike fertility and 1000-grain weight than *T. aestivum* L. cv. 'Rusałka'. The BC_1F_1 hybrid line was characterized by wheat-like fertility and phenotype. The F_5 hybrid lines were characterized by much higher variability of the analysed morphological traits than *T. aestivum* L. cv. 'Rusałka'. Grains of the hybrid lines had higher protein and micronutrient (iron and zinc) content than wheat grains.

The presence of DNA fragments specific to *Ae. kotschyi* Boiss. in the genotypes of the hybrid lines was confirmed by seven ISSR (Inter Simple Sequence Repeats) molecular markers. Two ISSR markers – $ISSR23_{690}$ and $ISSR33_{650}$ – were the most effective for germplasm analysis of the hybrid lines. The analysed lines can become a source material for improvement of common wheat *T. aestivum* L. in crossing programs.

Key words: *Aegilops kotschyi* Boiss., morphological traits, PCR ISSR, iron, zinc, protein, *Triticum aestivum* L.

INTRODUCTION

Intergeneric hybrids are interesting materials from a theoretical point of view and have the potential to increase genetic variation in common wheat breeding. The goal of distant hybridization is to introduce to breeding materials genes from wild species that code resistance to non-favourable agricultural conditions as well as high grain protein and micronutrient content. *Aegilops* species are often used as sources of desirable agronomic characters that can be introduced into wheat cultivars [1,2,3,4,5,6,7,8,9,10,11,12].

Aegilops kotschyi Boiss. (2n = 4x = 28, UUSS) is a species of particular interest for genetic and breeding research as an important gene donor for complex disease resistance [6,8,13,14,15], drought, heat and salt tolerance [4,16,17,18], because its grains have a high protein and lysine content [19,20,21]. Moreover, its cytoplasm is a potential basis for inducing cytoplasmic male sterility [22] and haploidy [23]. *Ae. kotschyi* Boiss. has higher iron and zinc content than *T. aestivum* L. and an efficient genetic system for uptake/translocation of the micronutrients, which could be effectively used for biofortification of wheat cultivars [24,25].

Wheat breeding programs have been directed towards factors such as grain yield and quality. An ideal cultivar for high grain yield or for any other desirable traits needs to express genetic potential with a low value of variance in different environmental factors of growing. Traits such as, for example, number of productive tillers, grain number and grain weight per spike, fertility of main spike, and 1000-grain weight were positively correlated with grain yield [17,26]. High temperature stress during the grain-filling period is one of the major environmental constraints limiting the grain yield of wheat in many countries in the world [27,17]. *Ae. kotschyi* Boiss. is the most xerophytic of the wild wheat relatives. Its genes can be used for genetic improvement of common wheat [28].

The basis of plant breeding is the selection of specific plants with desirable traits. Selection typically involves evaluating a breeding population for one or more traits in field or glasshouse trials (e.g. agronomic traits, disease resistance or stress tolerance), or with chemical tests (e.g. grain quality). The goal of plant breeding is to assemble more desirable combinations of genes in new varieties. In a commonly used pedigree breeding method, selecting desirable plants begins in early generations for traits of higher heritability. However, for traits of low heritability, selection is often postponed until the lines become more homozygous in later generations (F_5 or F_6). Selection of superior plants involves visual assessment for agronomic traits or resistance to stresses as well as laboratory tests for quality or other traits [29].

The aim of this study was to indicate diagnostic traits for the F_5 and BC_1 *Ae. kotschyi* Boiss. x *T. aestivum* L. hybrid lines which can be used in breeding programs for improvement of wheat grain yield and quality. Our interest focused on some agronomic characters such as morphological traits and yield components as well as grain protein, iron and zinc contents, and on the identification of genes of *Ae. kotschyi* Boiss. in the genotypes of the hybrid lines using the Inter Simple Sequence Repeats (ISSR) technique.

MATERIALS AND METHODS

A set of four F_5 *Aegilops kotschyi* Boiss. x *Triticum aestivum* L. cv. 'Rusałka' hybrid lines (KR3, KR4, KR6, KR9) and one BC_1 F_1 (*Ae. kotschyi* Boiss. x *T. aestivum* L. cv. Rusałka) x *T. aestivum* L. cv. 'Begra' hybrid line (KRB) originating from the crosses of *Ae. kotschyi* Boiss. no. AE 120/78 (seeds from the Institute of Plant Breeding in Cambridge, Great Britain) with *T. aestivum* L. cv. 'Rusałka' and *T. aestivum* L. cv. 'Begra' (seeds from the Institute of Genetics, Plant Breeding and Biotechnology, University of Life Sciences in Lublin, Poland) were used in this study. The 'Rusałka' cultivar was chosen for intergeneric crosses because it had good crossability with *Secale cereale* L.

The hybrid lines and their parental forms were cultivated under field conditions. 100 seeds of each hybrid line and cv. 'Rusałka' were sown manually in 1 m^2 plots, with 20 cm space between 2 m long rows and 10 cm distance between each seed in a row. The experiment was performed using a low seeding rate so that the hybrid lines could express their maximum genetic potential for tillering and other components. The fields were prepared with standard production practices. During the vegetation period, phenological observations were performed (emergence stage, tillering stage, winterhardiness, heading stage, flowering stage, maturity stage).

Analysis of quantitative morphological traits

For the analysis of morphological traits, 20 plants of each form at the full maturity stage were used. The following features were measured: productive tillering, length of the main tiller, diameter of the basal stem (the second internode from the bottom to the top of the plant), length of spike rachis, number of spikelets in the main spike, main spike density (the number of spikelets per 1 dm of spike rachis), grain number and weight per main spike, fertility of main spike (number of grains per spikelet), and 1000-grain weight.

Means and range of variation of agronomic characters were calculated. The significance of differences between the agronomic characters analysed were verified by Tukey's test at $p = 0.05$. The results are presented in Tables 1–2 and Figures 1–3.

Analysis of grain protein and micronutrient content

The protein content in grains of wheat and hybrid forms was determined by the Kjeldahl block digestion method [30]. For micronutrient analysis, the dried grain material was digested using a diacid (HNO_3-$HClO_4$) mixture. After dilution of the digests, they were processed for zinc (Zn) and iron (Fe) analysis using an atomic absorption spectrophotometer (AAS). The analysis of protein and micronutrient contents in grains was carried out in the Central Agro-Ecological Laboratory of the University of Life Sciences in Lublin.

Molecular analysis

DNA extraction from lyophilized leaves into a microfuge tube (1.5 mL) was done as in the modified Milligan [31] procedure. PCR ISSR analysis was performed by a modified procedure based on Ziętkiewicz et al. [32] for two DNA probes of each genotype, with a control reaction without DNA matrix performed at the same time. The 15 µL reaction volume contained 1 x PCR buffer (10 mM Tris-HCl pH 8.8, 50 mM KCl, 0.08% Nonidet P40, Fermentas, Lithuania), 130 µM of each dNTP, 470 pM of each primer, 1.5 mM $MgCl_2$, 0.5 unit of *Taq* DNA polymerase, and 60 ng template DNA. Of sixteen primers tested, seven were chosen for PCR ISSR analysis: ISSR 6: 5'– $(GT)_8C$ - 3', ISSR 14: 5'–$(GA)_7YG$ – 3', ISSR 16: 5'– $(GA)_8C$ – 3', ISSR 17: 5'– $(GA)_8YC$ – 3', ISSR 23: 5' – $(CA)_8GC$ – 3', ISSR 33: 5'– $(AG)_8T$ – 3', and ISSR 35: 5' – $(TC)_8CG$ – 3'.

DNA amplification reactions were performed in a thermocycler (Whatman-Biometra model T1) programmed as follows: an initial denaturation step at 95°C for 7 minutes followed by amplification for 38 cycles with denaturation at 95°C for 30 s, annealing the first 3 cycles at 54°C for 45 s, the following 3 cycles at 53°C for 45° and 32 cycles at 52°C for 45°, and extension at 72°C for 2 minutes with a final extension step at 72°C for 7 minutes. The amplified products were separated by electrophoresis in 2.5% agarose gel in 1 x TBE buffer, containing 0.01% ethidium bromide, in the presence of size markers. GeneRuler™ 100 bp DNA Ladder Plus (Fermentas, Lithuania) was used to determine the size of the products. DNA bands were photographed under ultra violet light, using a photo documentation system (version 3.03 of DNAfrag).

RESULTS

In eastern Poland conditions, the F_5 and BC_1F_1 *Ae. kotschyi* Boiss. x *T. aestivum* L. hybrid lines were characterized by winter hardiness and growing season length similar to those of cv. Rusałka.

Analysis of quantitative morphological traits

Table 1 contains means and range of variation of some morphological traits and yield components of the parental wheat *T. aestivum* L. cv. 'Rusałka' and the *Ae. kotschyi* Boiss. x *T. aestivum* L. hybrids lines. Cv. Rusałka was distinguished by lower tillering in comparison to the hybrid forms, except the KR 9 hybrid line. Cv. 'Rusałka' had a shorter length of main shoot and spike rachis than the studied hybrid lines. Some spikes of the KR3 hybrids were very long (1.8 dm) (Table 1, Fig. 1). The hybrid lines were distinguished by significantly lower main spike compactness in comparison to cv. 'Rusałka'.

The diameter of the second internode was similar in the case of all analysed forms. Only the KR3 hybrid line had a significantly lower number of spikelets in the main spike in comparison to cv. 'Rusałka'. Spikes of the hybrids were less dense than those of wheat. The F_5 hybrid lines (KR3, KR4, KR6, KR9) had a significantly lower number and weight of grains per main spike as well as lower main spike fertility and 1000-grain weight than cv. 'Rusałka'. In the case of 1000-grain weight of the KR 6 hybrid strain, this difference was not statistically significant. The KRB hybrid line had significantly lower grain weight per main spike and 1000-grain weight than wheat. But the number of grains per main spike and main spike fertility were similar to the wheat (Table 1).

In most of the F_5 *Ae. kotschyi* Boiss. x *T. aestivum* L. cv. 'Rusałka' hybrid lines, much higher variability of the analysed morphological traits was observed than in wheat cv. 'Rusałka' (Table 1). Grain number per spike is one of the most important yield components. The grain weight per main spike of the hybrid plants reached 4.6 g and the grain number reached 135. Grains of the F_5 and BC_1F_1 hybrid lines, despite their small weight, were characterized by rather good filling (Fig. 2).

Analysis of grain protein and micronutrient content

The grain protein content in the *Ae. kotschyi* Boiss. x *T. aestivum* L. hybrid strains varied from 17.6 to 23.5%, so it was higher than that in *T. aestivum* L. cv. 'Rusałka' (15.7%) (Table 1).

The grain iron and zinc concentrations in the hybrid lines were also analyzed and compared with *T. aestivum* L. cv. 'Rusałka' (Table 1). All the hybrids had higher iron and zinc content than wheat. The grain iron content in the *Ae. kotschyi* Boiss. x *T. aestivum* L. hybrid lines varied from 46.5 to 54.0 mg × kg^{-1} DW, whereas in the case of zinc – from 39.8 to 57.8 mg × kg^{-1} DW. It was more than in the case of grains of *T. aestivum* L. cv. 'Rusałka' (41.7 mg × Fe kg^{-1} DW and 23.6 mg × Zn kg^{-1} DW, respectively).

Molecular analysis

The hybrid nature of the *Ae. kotschyi* Boiss. x *T. aestivum* L. lines was confirmed through DNA analysis. The presence of markers specific for *Ae. kotschyi* Boiss. in the genotypes of the *Ae. kotschyi* x *T. aestivum* L. cv. 'Rusałka' and (*Ae. kotschyi* Boiss. x *T. aestivum* L. cv. 'Rusałka') x *T. aestivum* L. cv. 'Begra' hybrid lines was confirmed using the PCR ISSR method.

In our study, based on electrophoresis of PCR amplification products, the presence of *Ae. kotschyi* Boiss. ISSR markers was proved in the case of all *Ae. kotschyi* Boiss. x *T. aestivum* L. hybrids (Table 2, Fig. 3). The $ISSR23_{690}$ and $ISSR33_{650}$ markers were detected in the greatest number of hybrids. The $ISSR23_{690}$ marker was detected in eight hybrids and $ISSR33_{650}$ in six. The $ISSR6_{990}$ and $ISSR33_{380}$ polymorphic bands were observed in four hybrids, and $ISSR35_{2800}$ and $ISSR35_{2600}$ in three. The remaining markers were present in one or two hybrid lines. The biggest number of ISSR markers was found in the F_5 KR 3 and F_4 KR 4 hybrid lines (9 markers). Slightly fewer ISSR polymorphic bands appeared in the F_4 KR 3 (6 markers), F_5 KR 6 (5 markers), F_5 KR 4 (4 markers) and F_2 KRB lines (4 and 3 markers). One polymorphic band specific for *Ae. kotschyi* Boiss. was noted in the F_4 KR 6 and F_4 KR 9 lines. No markers were detected in case of the F_5 KR 9 line.

Table 1

Mean values and range of variation of some morphological traits and protein, iron and zinc contents in the grains of F_5 and BC_1F_1 *Aegilops kotschyi* Boiss. x *Triticum aestivum* L. hybrid lines and *Triticum aestivum* L. cv. 'Rusałka'

Forms analysed	Productive tillering	Length of main shoot (cm)	Diameter of 2nd bottom internode (mm)	Length of spike rachis (dm)	No. of spikelets in main spike	Main spike compactness	No. of grains per main spike	Weight of grains per main spike (g)	Main spike fertility	1000-grain weight (g)	Total protein content (%)	Iron content (mg × kg^{-1} DW)	Zinc content (mg × kg^{1} DW)
KR 3 *Ae. kotschyi* x Rusałka	29.6* (8.0-50.0)	95,5* (46.0-120.0)	4.2 (2.7-5.3)	1.2* (0.7-1.8)	17.2* (15.0-19.0)	13.6* (9.1-20.0)	9.1* (1.0-26.0)	0.3* (0.01-1.2)	0.5* (0.06-1.37)	25.0* (11.0-40.8)	23.5	54.0	49.6
KR 4 *Ae. kotschyi* x Rusałka	20.1* (4.0-41.0)	88.7 (49.0-111.0)	4.5 (3.4-5.9)	1.1 (0.6-1.3)	18.3 (14.0-26.0)	16.5* (13.1-23.8)	20.9* (0.0-58.0)	0.7* (0.0-2.4)	1.1* (0.0-3.2)	28.3* (0.0-43.0)	23.0	47.8	45.1
KR 6 *Ae. kotschyi* x Rusałka	19.0 (6.0-34.0)	97.5* (62.0-131.0)	4.2 (3.6-4.9)	1.1 (0.9-1.2)	19.8 (18.0-22.0)	17.9 (14.8-21.0)	17.0* (8.0-25.0)	0.6* (0.2-1.0)	0.8* (0.4-1.4)	28.2 (19.3-37.4)	21.7	50.4	42.5
KR 9 *Ae. kotschyi* x Rusałka	9.7 (5.0-12.0)	80.7 (73.0-86.0)	4.7 (4.3-5.1)	0.9 (0.8-1.1)	17.0 (15.0-20.0)	17.2* (15.8-18.7)	5.0* (0.0-14.0)	0.2* (0.0-0.6)	0.3* (0.0-0.7)	12.8* (0.0-26.3)	18.5	46.5	57.8
KRB (*Ae.kotschyi* x Rusałka) x Begra	19.3* (14.0-24.0)	96.3* (91.0-103.0)	5.3 (4.70-5.70)	1.2* (1.1-1.2)	22.3 (20.0-24.0)	18.0 (16.5-18.3)	47.3 (37.0-60.0)	1.3* (0.8-1.9)	2.25 (1.9-2.6)	24.4* (19.2-27.3)	17.6	48.6	39.8
Rusałka	12.7 (5.0-22.0)	77.5 (64.0-94.0)	4.5 (3.5-6.0)	0.88 (0.60-1.20)	19.1 (16.0-22.0)	21.0 (15.8-28.3)	38.1 (16.0-52.0)	2.1 (0.8-3.0)	2.0 (0.8-2.9)	41.6 (25.4-67.5)	15.7	41.7	23.6

* result significantly different in relation to the wheat at p=0.05

Table 2

Specification of presence in the genotypes of *Ae. kotschyi* Boiss. x *T. aestivum* L. cv. Rusałka (KR) and (*Ae. kotschyi* Boiss. x *T. aestivum* L. cv. 'Rusałka') x *T. aestivum* L. cv. Begra (KRB) hybrid lines specific for *Ae. kotschyi* Boiss. ISSR markers

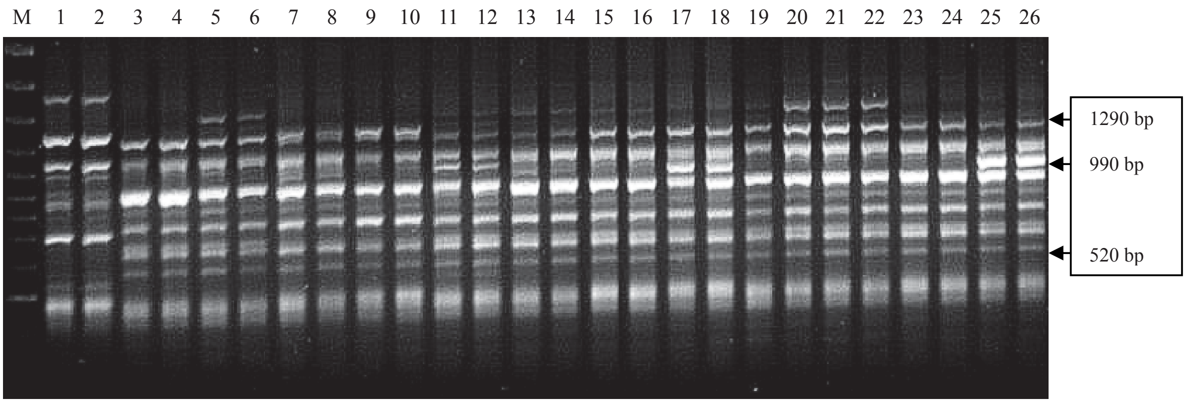
			Forms analysed												
No.	Primer	Size of fragment (bp)*	*Ae. kotschyi*	Rusałka	Begra	F_4 KR 3	F_5 KR 3	F_4 KR 4	F_5 KR 4	F_4 KR 6	F_5 KR 6	F_4 KR 9	F_5 KR 9	F_2 KRB	F_2 KRB
1	ISSR6	1290	+			+	+								
		990	+			+		+			+				+
		520	+					+							
2	ISSR14	1010	+				+	+							
		340	+					+			+				
		280	+			+	+								
3	ISSR16	555	+				+								
4	ISSR17	350	+				+		+						
5	ISSR23	690	+			+	+	+	+	+	+			+	+
		455	+					+							
6	ISSR33	1360	+					+							
		650	+			+	+	+	+					+	+
		380	+					+	+					+	+
7	ISSR35	2800	+				+				+	+			
		2600	+			+	+				+				
	Total of fragments		15	0	0	6	9	9	4	1	5	1	0	3	4
	Hybrid verification					+	+	+	+	+	+	+		+	+

* - base pairs

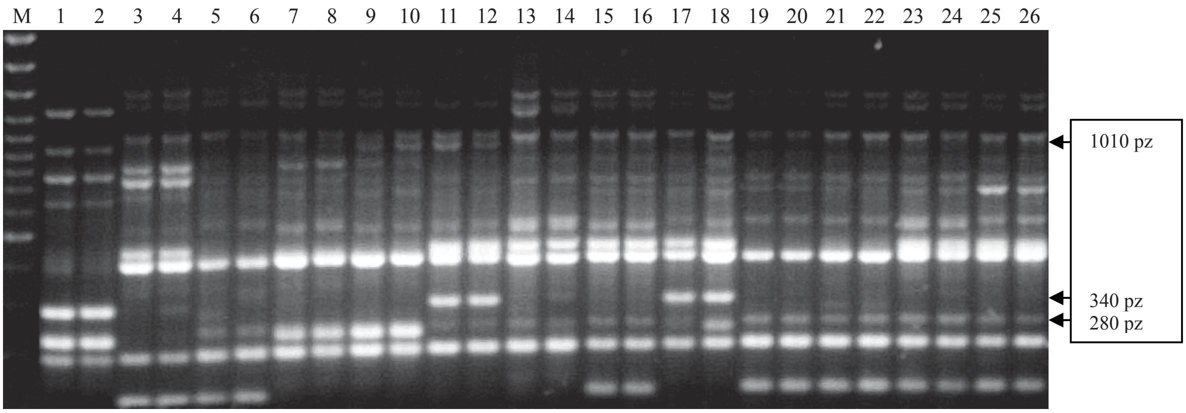
Fig. 1. Spikes (from the left): 1–7, 11–12 F_5 hybrid lines of *Ae. kotschyi* Boiss. x *T. aestivum* L. cv. 'Rusałka', 8–10 BC_1 hybrid line of (*Ae. kotschyi* Boiss. x *T. aestivum* L. cv. 'Rusałka') x *T. aestivum* L. cv. 'Begra', *Ae. kotschyi* Boiss., *T. aestivum* L. cv. 'Rusałka'

Fig. 2. Grains (from the left): upper row 1–3 F_5 hybrid lines of *Ae. kotschyi* Boiss. x *T. aestivum* L. cv. 'Rusałka', lower row 1–3 BC_1 hybrid line of (*Ae. kotschyi* Boiss. x *T. aestivum* L. cv. 'Rusałka') x *T. aestivum* L. cv. 'Begra', *T. aestivum* L. cv. 'Rusałka'

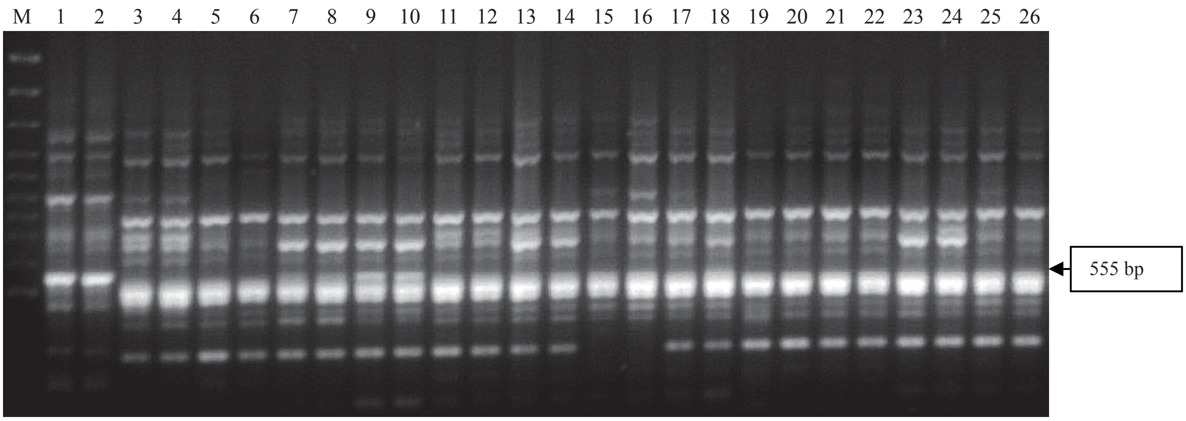
ISSR6



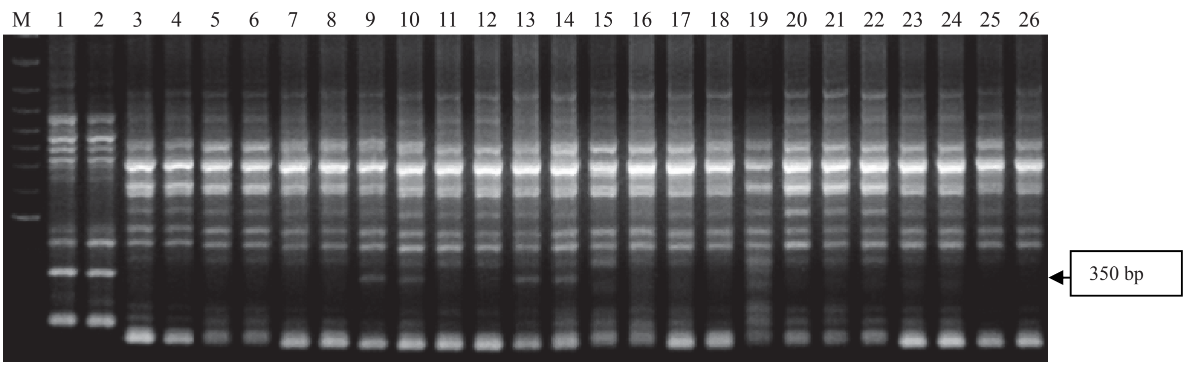
ISSR14



ISSR16



ISSR17



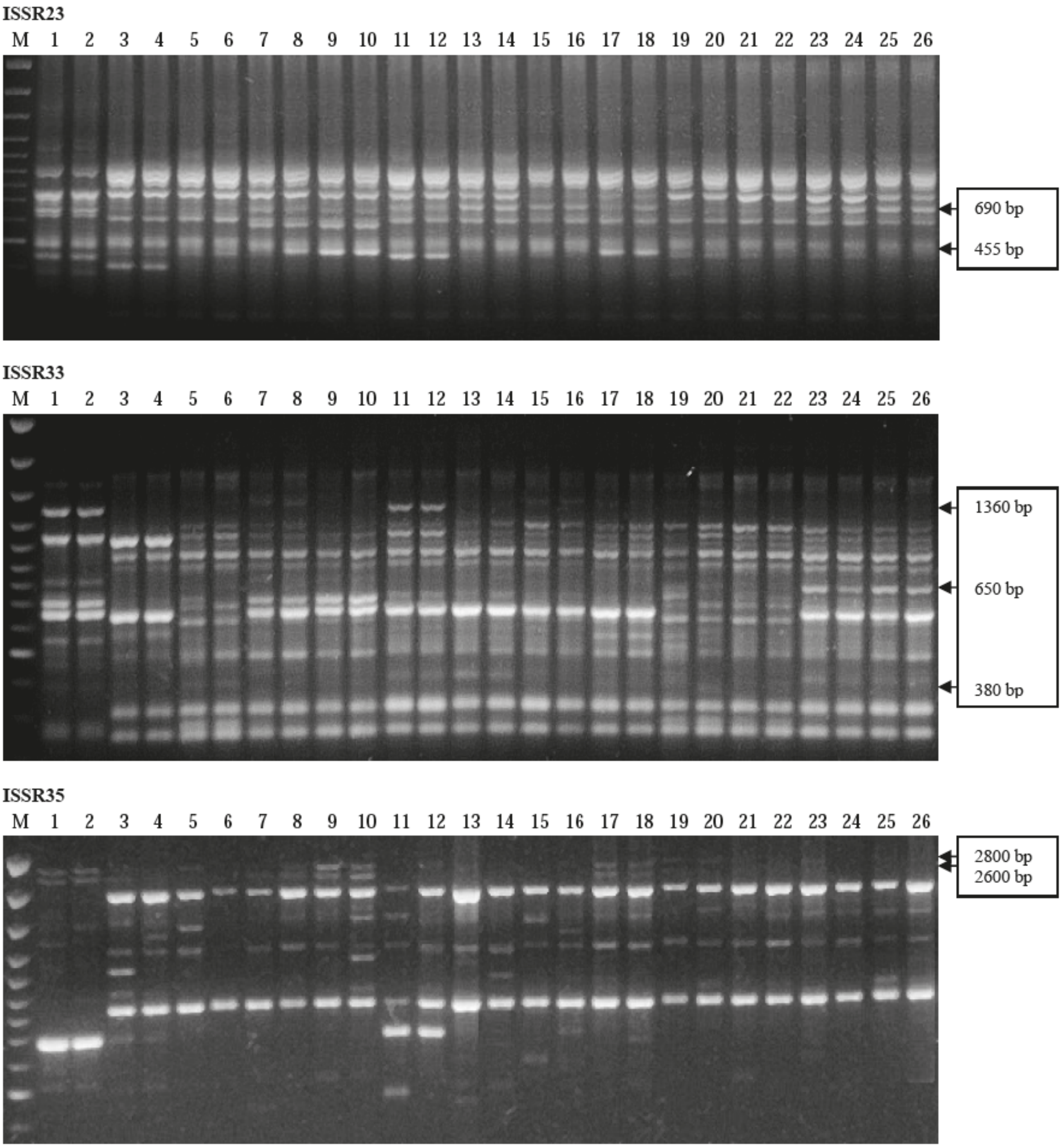


Fig. 3. Gel pictures of ISRR with primers 6, 14, 16, 17, 23, 33, 35 of *Ae. kotschyi* Boiss., *T. aestivum* L., KR *Ae. kotschyi* Boiss.x cv. 'Rusałka' and KRB (*Ae. kotschyi* Boiss. x cv. 'Rusałka') x cv. 'Begra' hybrid lines (arrows show *Ae. kotschyi* Boiss. markers): M) marker GeneRuler^{TM} 100bp DNA Ladder Plus, 1–2) *Ae. kotschyi* Boiss., 3–4) cv. 'Rusałka', 5–6) cv. 'Begra', 7–8) F_4 KR3, 9–10) F_5 KR3, 11–12) F_4 KR4, 13–14) F_5 KR4, 15–16) F_4 KR6, 17–18) F_5 KR6, 19–20) F_4 KR9, 21–22) F_5 KR9, 23–24) F_2 KRB, 25–26) F_2 KRB

DISCUSSION

Among the evaluated F_5 hybrids, the most distinguished was the KR3 *Ae. kotschyi* Boiss. x *T. aestivum* L. cv. 'Rusałka' line due to the highest length of spike rachis, and grain protein and micronutrient content. In this line, the largest number of ISSR markers specific for *Ae. kotschyi* Boiss. was found (9 markers).

Generally, the *Ae. kotschyi* x *T. aestivum* L. hybrid lines were characterized by more productive tillers, longer shoots and spike rachises as well as lower main spike compactness, number and weight of grain per main spike, fertility, and 1000-grain weight in comparison to cv. 'Rusałka'. The values of the quantitative traits in the F_5 hybrid lines varied and these lines were characterized by much higher variability of the analyzed morphological traits than cv. 'Rusałka'. This research is in agreement with previously published papers which also found high variability in yield components of *Aegilops* L. x wheat hybrids. In the studies conducted by Stefanowska

[33] as well as by Tyrka and Stefanowska [34], *Aegilops* L. x *Triticum* L. hybrids were characterized by high variability of the analyzed morphological traits in comparison to common wheat. The length of the main shoot of *Ae. juvenalis* (Thell.) Eig. and *Ae. ventricosa* Tausch. with *Triticum* sp. hybrids ranged from 65.4 to 118.8 cm, the diameter of the second internode from 2.5 to 4.4 mm, the number of spikelets in the main spike from 9.9 to 23.0, and 1000-grain weight from 21.6 to 52.1 g. In the present study, the length of the main shoot of the *Ae. kotschyi* Boiss. x *T. aestivum* L. hybrid lines ranged from 80.7 to 97.5 cm, the diameter of the second internode from 4.2 to 5.3 mm, the number of spikelets in the main spike from 17.0 to 22.3, and 1000-grain weight from 12.8 to 28.3 g. Pilch and Głowacz [10] found 28 spikelets in the main spike of *Ae. speltoides* Tausch. x *T. aestivum* L. hybrids. These authors also reported that some of the hybrid forms significantly exceeded wheat with respect to number and weight of grains per main spike. *Aegilops* L. x *Triticum* L. hybrid lines selected by Pilch [35] were characterized by long spikes with large grains, much bigger than those of wheat *T. aestivum* L. cv. 'Chinese Spring'.

In the present study, spikes of the F_5 *Ae. kotschyi* Boiss. x *T. aestivum* L. hybrid lines were less different in density, awning, shape, and length of spike rachis than spikes of the F_4 hybrids [36].

Blüthner and Schumann [19] reported that the grain protein content in *Ae. kotschyi* Boiss. was 27.70%. Rawat et al. [25] noted that BC_2 and BC_1F_2 plants from interspecific crosses between 'Chinese Spring' CS (Ph') wheat and *Ae. kotschyi* Boiss. had a higher level of grain iron and zinc concentrations (26.8–79.8 and 22.1–55.2 mg kg^{-1} DW, respectively), similarly to our study. Cakmak et al. [37] also indicated lower iron and zinc content among *T. aestivum* cultivars as compared to wild and primitive *Triticum* species. The wild relatives of wheat, especially *Ae. kotschyi* Boiss., and other S genome *Aegilops* species are a promising source for enriching cultivated wheats to obtain a high Fe and Zn content [24]. *Ae. kotschyi* Boiss. is easily crossable with hexaploid wheat and thus can be used for transferring high Fe and Zn content to cultivated wheats through the induction of homoeologous pairing [24]. In addition to the S genome, some other gnomes (U, M) of *Aegilops s*pecies also control high iron and zinc content, which can be exploited for biofortification. Biofortification is a method of breeding crops to increase their nutritional value [37].

DNA markers are used to select and identify desirable forms, to assess the adjustment of breeding material, to confirm crossbreeding efficiency, and to identify the genes which determine important functional traits [38]. In the present study, ISSR markers were used to identify the genes of *Ae. kotschyi* Boiss. in the genotypes of the hybrids lines. In our study, the $ISSR23_{690}$ and $ISSR33_{650}$ markers were detected in the largest number of the hybrids (8 and 6).

The inter simple sequence repeats (ISSR) are a kind of molecular marker involving PCR amplification of DNA by a single primer 16–18 bp long composed of a repeated sequence anchored at the 3' or 5' end by 2–4 arbitrary nucleotides [32]. They are easy to handle, highly informative and repeatable. Since repeated sequences are abundant throughout the genome, SSR primers anneal in several regions, typically giving a complex amplification pattern in which fragments are often polymorphic between different individuals. Among the various available molecular markers, microsatellites have been shown to be superior to other DNA-based markers because of their higher level of polymorphism and informativeness in hexaploid wheat [39, 40, 41]. Microsatellite markers were used for germplasm analysis and estimation of the genetic relationship between accessions of *Ae. tauschii* Coss. [42]. Microsatellite analysis of germplasm is also useful in molecular investigations of *Aegilops* species and their hybrids. Galaev et al. [43] detected the introgression of genome elements of the *Ae. cylindrica* Host into the *T. aestivum* L. genome by ISSR analysis. Grądzielewska et al. [44] estimated the genetic similarity in triticale (6x) hybrids with *Ae. crassa* (4x) Boiss and confirmed the existence of DNA fragments of the wild species in the triticale (6x) hybrid strains background. In the analyses, fourteen ISSR selected primers were used. Huguet-Robert et al. [45] used microsatellite markers to locate the gene *Ph 1* for resistance to eyespot in the resistant *Ae. ventricosa* Tausch. x *T. durum* Desf. cv. Creso recombination lines. Kuraparthy et al. [9] analysed wheat-alien translocation, with leaf rust and stripe rust resistance genes *Lr57* and *Yr40* transferred from *Ae. geniculata* Roth., using physically mapped ESTs. Microsatellite markers linked to the *Pm* gene of *Ae. tauschii* Coss. introgressed into common wheat were identified by Miranda et al. [46].

CONCLUSIONS

1. Most of the *Aegilops kotschyi* Boiss. x *T. aestivum* L. hybrid lines were observed to have more productive tillers, longer shoots and longer, more loose spikes as well as lower number and weight of grains per main spike, fertility and 1000-grain weight than cv. 'Rusałka',
2. The majority of the hybrid lines were characterized by much higher variability of the analysed morphological traits than the wheat cultivar 'Rusałka'.

4. The hybrid lines contained in grains more protein and micronutrients – iron and zinc – in comparison to the 'Rusałka' cultivar. Among the hybrid lines, KR3 *Ae. kotschyi* Boiss. x *T. aestivum L.* cv. 'Rusałka' was distinguished by the highest grain protein, iron and zinc content.
5. The PCR ISSR method confirmed the presence of markers specific for *Ae. kotschyi* Boiss. in the hybrid genotypes. Among the ISSR markers analysed, the most successful were $ISSR23_{690}$ and $ISSR33_{650}$.

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Authors' contributions

The following declarations about authors' contributions to the research have been made: study conception: RP; collecting data: RP; morphological data analysis and interpretation: RP; protein and micronutrients analyses – data interpretation: RP; molecular analysis and data interpretation: EP-G; writing the manuscript, table and figure arrangement: RP, EP-G.

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Charakterystyka linii mieszańcowych *Aegilops kotschyi* Boiss. x *Triticum aestivum* L.

Streszczenie

Przeprowadzono ocenę cech morfologicznych i jakościowych czterech linii mieszańcowych F_5 i jednej BC_1F_1 *Aegilops kotschyi* Boiss. x *Triticum aestivum* L. Linie mieszańcowe wykazały się pośrednimi wartościami cech morfologicznych w porównaniu do *Aegilops kotschyi* Boiss. i *Triticum aestivum* L. lub zbliżonymi do jednej z form rodzicielskich. Linie mieszańcowe F_5 miały istotnie niższą liczbę i masę ziarniaków z kłosa, płodność kłosa i masę tysiąca ziarniaków od pszenicy *T. aestivum* L. odmiany 'Rusałka'. Linia mieszańcowa BC_1F_1 charakteryzowała się podobną do pszenicy płodnością i fenotypem. Zmienność cech morfologicznych linii mieszańcowych F_5 była znacznie większa niż pszenicy odmiany 'Rusałka'. Ziarniaki linii mieszańcowych zawierały więcej białka i mikroelementów (żelaza i cynku) niż ziarniaki pszenicy.

W liniach mieszańcowych potwierdzono obecność prążków specyficznych dla *Ae. kotschyi* Boiss. za pomocą siedmiu markerów molekularnych ISSR (Inter Simple Sequence Repeats – polimorfizm odcinków DNA pomiędzy mikrosatelitami). Dwa markery ISSR – $ISSR23_{690}$ i $ISSR33_{650}$ – okazały się najbardziej efektywne w analizie genomów linii mieszańcowych. Badane linie mogą zostać wykorzystane w programach hodowlanych mających na celu ulepszenie pszenicy zwyczajnej.

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