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Aegilops-Secale amphiploids: chromosome categorisation, pollen viability and identification of fungal disease resistance genes

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Abstract The aim of this study was to assess the potential breeding value of goatgrass-rye amphiploids, which we are using as a "bridge" in a transfer of *Aegilops* chromatin (containing, e.g. leaf rust resistance genes) into triticale. We analysed the chromosomal constitution (by genomic in situ hybridisation, GISH), fertility (by pollen viability tests) and the presence of leaf rust and eyespot resistance genes (by molecular and endopeptidase assays) in a collection of 6× and 4× amphiploids originating from crosses between five *Aegilops* species and *Secale cereale*. In the five hexaploid amphiploids *Aegilops kotschyi* × *Secale cereale* (genome UUSSRR), *Ae. variabilis* × *S. cereale* (UUSSRR), *Ae. biuncialis* × *S. cereale* (UUMMRR; two lines) and *Ae. ovata* × *S. cereale* (UUMMRR), 28 *Aegilops* chromosomes were recognised, while in the *Ae. tauschii* × *S. cereale* amphiploid (4×; DDRR), only 14 such chromosomes were identified. In the materials, the number of rye chromosomes varied from 14 to 16. In one line of *Ae. ovata* × *S. cereale*, the U-R translocation was found. Pollen viability varied from 24.4 to 75.4%. The leaf rust resistance genes *Lr22*, *Lr39* and *Lr41* were identified in *Ae. tauschii* and the 4× amphiploid *Ae. tauschii* × *S. cereale*. For the first time, the leaf rust resistance gene *Lr37* was found in *Ae. kotschyi*, *Ae. ovata*, *Ae. biuncialis* and amphiploids derived from those parental species. No eyespot resistance gene *Pch1* was found in the amphiploids.

Keywords *Aegilops-Secale* · Amphiploids · Eyespot · Genomic in situ hybridisation · Leaf rust · Resistance genes

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Fungal diseases of triticale, such as leaf rust (caused by *Puccinia triticina*) and eyespot (caused by *Oculimacula yallundae* and *O. acuformis*), have escalated recently. The introgression of effective resistance genes from wild species, like *Aegilops* spp., is a way to increase the genetic diversity of triticale. Goatgrasses (*Aegilops* spp.), the wild relatives of wheat and triticale, are a rich source of novel genes for resistance to various pathogens (Gill et al. 1985; Molnár et al. 2005; Prażak 2007; Schneider et al. 2008). *Aegilops* spp. carry many resistance genes to biotic factors: rusts (Dhaliwal et al. 2002), powdery mildew (Miranda et al. 2007) and eyespot (Leonard et al. 2008), and abiotic factors: drought (Baalbaki et al. 2006) and salinity (Landjeva and Ganeva 1999). The goatgrass-rye amphiploids can be used as a "bridge" to transfer useful agronomic traits from *Aegilops* spp. to cultivated cereals, like triticale and rye (Wojciechowska and Pudelska 2005).

This research is a first step to increase the genetic diversity of cultivated triticale (Polish cultivars). The major aims of this work were: (1) to analyse the chromosomal constitution of amphiploids by genomic in situ hybridisation (GISH); (2) to evaluate their fertility by pollen viability tests; and (3) to identify leaf rust and eyespot resistance genes in the amphiploids.

Six amphiploid lines, *Ae. kotschyi* × *S. cereale* (UUSSRR), *Ae. variabilis* × *S. cereale* (UUSSRR), *Ae. biuncialis* × *S. cereale* (UUMMRR, two lines), *Ae. ovata* × *S. cereale*

Table 1 Chromosomal constitution in the analysed amphiploids (assessed by genomic in situ hybridisation, GISH) and their pollen viability

Aegilops-Secale amphiploids	2*n*	Number of chromosomes		Pollen viability (%)
		Aegilops spp.	*S. cereale*	
Ae. kotschyi (UUSS) × *S. cereale* (RR)	42–44	28 [14U + 14S]	14–16 [R]	65.7
Ae. variabilis (UUSS) × *S. cereale* (RR)	40–42	28 [14U + 14S]	12, 14 [R]	70.6
Ae. biuncialis (line 1) (UUMM) × *S. cereale* (RR)	42	28 [14U + 14M]	14 [R]	75.4
Ae. biuncialis (line 2) (UMM) × *S. cereale* (RR)	42	28 [14U + 14M]	14 [R]	24.4
Ae. ovata (UUMM) × *S. cereale* (RR)	42	28 [14*U + 14M]	14* [R]	64.4
Ae. tauschii (DD) × *S. cereale* (RR)	28	14 [D]	14 [R]	42.1

*In one of the *Ae. ovata* × *S. cereale* amphiploids (plant 3/4), an introgression of *Ae. ovata* chromatin in the telomeric region of rye chromosomes was observed

(UUMMRR) and *Ae. tauschii* × *S. cereale* (DDRR), were provided by the Institute of Plant Genetics, Polish Academy of Sciences (Poznań, Poland). In this study, 20 plants of each line were used.

For the GISH analysis, seeds of the 20 plants of each amphiploid line were germinated on moist filter paper in Petri dishes. The root-tip chromosome preparations were made according to Pijnacker and Ferwerda (1984). The GISH procedure was carried out according to Schwarzacher and Heslop-Harrison (2000), with modifications, using the genomic DNA of *Aegilops* spp., labelled (according to the standard oligolabelling protocol) with digoxigenin-11-dUTP (Roche) as a probe. Unlabelled genomic DNA from rye (*S. cereale* cv. Dańkowskie Złote) was sheared by autoclaving and used as a block. The slides were counter-stained with PI or DAPI in Vectashields. An Olympus BX 60 epifluorescence microscope fitted with a CCD camera was used to supply documentary evidence.

The number of *Aegilops* chromosomes was constant and amounted to 28 chromosomes in *Ae. kotschyi* × *S. cereale*, *Ae. biuncialis* (line 1) × *S. cereale*, *Ae. biuncialis* (line 2) × *S. cereale*, *Ae. variabilis* × *S. cereale*, *Ae. ovata* × *S. cereale* and 14 chromosomes in *Ae. tauschii* × *S. cereale* amphiploids. The number of identified rye chromosomes was also constant (14 chromosomes) in three amphiploids: *Ae. biuncialis* (line 1) × *S. cereale*, *Ae. biuncialis* (line 2) × *S. cereale* and *Ae. tauschii* × *S. cereale*. However, the *Ae. ovata* × *S. cereale* and *Ae. kotschyi* × *S. cereale* amphiploids had 14 to 16 rye chromosomes, while the *Ae. variabilis* × *S. cereale* amphiploids had 12 or 14 rye chromosomes (Table 1). In one of the *Ae. ovata* × *S. cereale* plants, an introgression of the *Ae. ovata* chromatin in the telomeric region of a rye chromosome was observed (Figs. 1, 2).

Multi-colour GISH was also applied to distinguish subgenomic (U,S,M) chromosomes of the *Aegilops* spp. studied. The total genomic DNA of *Ae. umbellulata* (UU), *Ae. sharonensis* (SS) and *Ae. comosa* (MM) was labelled (according to the standard oligolabelling protocol) with digoxigenin-11-dUTP or rhodamine-4-dUTP (Roche) and used as a probe. The GISH protocol was analogous to previous researches. The number of each subgenomic chromosome in a given amphiploid combination (i.e. UU and MM or UU and SS) was constant and amounted to 14. No translocations were identified with one exception (plant no. 3/4 of *Ae. ovata* × *S. cereale*), where the introgression

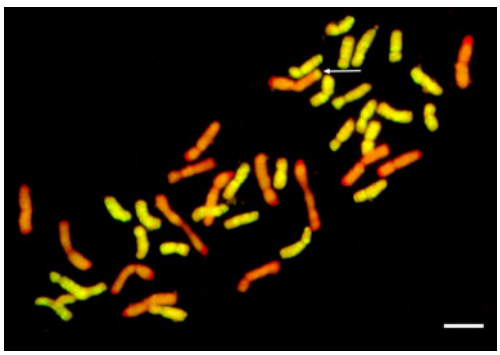

Fig. 1 Genomic in situ hybridisation (GISH) discrimination of the U°M° genome (*green*) and rye chromosomes (*red*) in a root-tip cell at mitotic metaphase in an *Ae. ovata* × *S. cereale* amphiploid. Introgression of the *Ae. ovata* chromatin in the telomeric region of a rye chromosome (*arrow*). Scale bar=10 μm

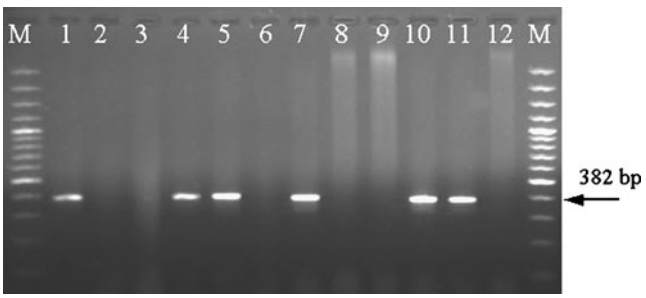

Fig. 2 Amplified products for the *CslVrgal3'F/R* marker (*Lr37*). (M) DNA size ladder, (1) *Ae. kotschyi*, (2) *Ae. variabilis*, (3) *Ae. biuncialis* (line 2) (4) *Ae. ovata*, (5) *Ae. biuncialis* (line 1), (6) *Ae. tauschii*, (7) *Ae. kotschyi* × *S. cereale*, (8) *Ae. variabilis* × *S. cereale*, (9) *Ae. biuncialis* (line 1) × *S. cereale*, (10) *Ae. ovata* × *S. cereale*, (11) *Ae. biuncialis* (line 2) × *S. cereale*, (12) *Ae. tauschii* × *S. cereale*. Amplified bands (382 bp) show the presence of *Lr37* and indicate leaf rust resistance

of *Ae. umbellulata* chromatin in the *S. cereale* chromosome was observed.

Pollen viability was measured as pollen stainability (%). For each of the 120 plants, four preparations were made by pollen staining with Fulgen liquid. As shown in Table 1, pollen stainability was the highest in *Ae. biuncialis* (line 1) × *S. cereale* (75.4%), while it was the lowest in *Ae. biuncialis* (line 2) × *S. cereale* (24.4%).

The identification of 12 leaf rust resistance genes (*Lr9*, *Lr10*, *Lr19*, *Lr20*, *Lr22*, *Lr28*, *Lr29*, *Lr35*, *Lr37*, *Lr39*, *Lr41* and *Lr47*) was made by using molecular markers according to Błaszczyk et al. (2004, 2008), as well as Chełkowski et al. (2003). Leaf tissues from amphiploids and *Aegilops* lines (donors of wild chromatin) were sampled three weeks after planting, freeze-dried and used for DNA extraction according to a CTAB-based procedure (Doohan et al. 1998). The leaf rust resistance genes *Lr22*, *Lr39* and *Lr41* were identified in *Ae. tauschii* and amphiploids: *Ae. tauschii* × *S. cereale*, which was consistent with previous reports on the location of *Lr22*, *Lr39* and *Lr41* in 2DS (Dyck 1979), 2DS (Raupp et al. 2001) and 1D (Singh et al. 2004), respectively. Three lines of *Aegilops* spp., *Ae. kotschyi*, *Ae. ovata* and *Ae. biuncialis* (line 1), and amphiploids derived from their hybridisation with rye, were found to carry the *Lr37* gene, which is novel (Fig. 2). Previously, the presence of *Lr37* was only reported in chromosome 2NS of *Ae. ventricosa* (Helguera et al. 2003). However, there was no information about other *Aegilops* spp. carrying this gene.

The identification of *Pch1* eyespot resistance genes was made by using endopeptidase assays and molecular markers (*Xust SSR2001-7DL*), according to Groenewald et al. (2003) and Santra et al. (2006). No *Pch1* eyespot resistance genes in the *Aegilops* lines and in the amphiploids were detected.

In conclusion, the GISH method confirmed the cytogenetic stability of the used amphiploids, and their fertility assessed as pollen viability is also acceptable (with the exception of *Ae. variabilis* × *S. cereale*). The results showed that the examined amphiploids could serve as a starting material for the transfer of *Aegilops* chromatin to cultivated triticale. Furthermore, the amphiploids with transferred leaf rust resistance genes (*Lr22*, *Lr39*, *Lr41* and *Lr37*) from *Aegilops* donors appear to be attractive forms, which can be exploited in future breeding programmes dealing with a widening of genetic diversity in the Triticeae. Given that the *Lr37* gene is closely associated with the yellow rust resistance gene *Yr17* and the stripe rust resistance gene *Sr38* in *Ae. ventricosa* (Seah et al. 2001), it is useful to check the presence of *Yr17* and *Sr38* in the analysed *Ae. kotschyi*, *Ae. ovata* and *Ae. biuncialis* (line 1). The transfer of the *Aegilops* chromatin by using the described amphiploids (with the leaf rust resistance genes present in the given amphiploids) to cultivated triticale and chromosome identification using fluorescence in situ hybridisation (FISH) with repetitive DNA probes will be the next steps of this research.

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