***Roegneria alashanica* Keng: a species with the StStSt^YSt^Y genome constitution**

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Roegneria alashanica Keng: a species with the **StStSt^YSt^Y** genome constitution

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Abstract

The genome constitution of tetraploid *Roegneria alashanica* Keng has been in question for a long time. Most scientific studies have suggested that *R. alashanica* had two versions of the **St** genome, **St₁St₂**, similar to that of *Pseudoroegneria elytrigioides* (C. Yen et J. L. Yang) B. R. Lu. A study, however, concluded that *R. alashanica* had the **StY** genome formula typical for tetraploid *Roegneria* species. For the present study, *R. alashanica*, *Elymus longearistatus* (Bioss.) Tzvelev (**StY**-genomes), *Pseudoroegneria strigosa* (M. Bieb.) Á. Löve (**St**), *P. libanotica* (Hackel) D.R. Dewey (**St**), and *P. spicata* (Pursh) Á. Löve (**St**) were screened for the **Y**-genome specific marker, B14(F+R)₂₆₉. All *E. longearistatus* plants expressed intense bands specific to the **Y** genome. Only 6 of 10 *R. alashanica* plants exhibited relatively faint bands for the STS marker. Previously, the genome in *Pseudoroegneria* species exhibiting such faint **Y**-genome specific marker was designated as **St^Y**. Based on these results, *R. alashanica* lacks the **Y** genome in *E. longearistatus* but likely possess two remotely related **St** genome, **St** and **St^Y**. According to its genome constitution, *R. alashanica* should be classified in the genus *Pseudoroegneria* and given the new name, *Pseudoroegneria alashanica* (Keng) R. R.-C. Wang and K. B. Jensen.

Keywords: Triticeae, *Pseudoroegneria*, taxonomy, nomenclature.

Introduction

The taxonomy and nomenclature of the tribe Triticeae, which contains many important North American and Asian cool-season forage grasses, has been and continues to be in a state of taxonomic uncertainty (Jensen and Anderson 2014; Wang and Lu 2014). In the broad-sense, *Elymus* L. is comprised of approximately 150 perennial species with the largest geographical distribution, where it extends from North America into Europe, South America, and Australia (Barkworth et al. 2007). The *Elymus* complex is comprised of allopolyploids with chromosome numbers ranging from $2n=4x=28$ to $2n=8x=56$ (Liu et al. 2006). Genome combinations occurring within *Elymus* species include **StH**, **StP**, **StY**, **StStY**, **StHY**, **StYW**, and **StYP** (Dewey 1984; Baum et al. 1991; Wang et al. 1995; Larson et al. 2003; Yen and Yang 2009), where **St** genome came from the genus *Pseudoroegneria* (Nevski) Á. Löve (Dewey 1980; Wang et al. 1995), **H** genome from *Hordeum* L., **P** from *Agropyron* J. Gaertn., and **W** from *Australopyrum* (Tzvelev) Á. Löve. To date, the donor of the **Y** genome present in the majority of the Asiatic *Elymus* species has not yet been identified (Wang et al. 1995; Wang and Lu 2014). Yen et al. (2005a) suggested that *Elymus* s.l. be divided into *Elymus sensu stricto* (**StH** genomes), *Roegneria* (**StY** genomes), *Anthosachne* (**StYW** genomes), *Campeioestachys* (**StYH** genomes), and *Kengyilia* (**StYP** genomes). Yen et al. (2005b) named another genus *Douglasdeweya* to accommodate species with the **StP** genome combination.

When originally described by Koch (1848), the genus *Roegneria* was comprised of *R. caucasica* C. Koch as a monotypic genus. Many of the species treated previously in *Agropyron* and *Elymus* are now recognized in the genus *Roegneria* (Baum et al. 1991). Morphologically, Baum et al. (1991) concluded that *Roegneria* was difficult to differentiate from *Agropyron* and *Elymus*. Defined genomically, *Roegneria* species contain both the **St** and **Y** genomes. Based on

bivalent chromosome pairing in allotetraploid **StY** species, Dewey (1980) and others (Jensen 1990, Torabinejad and Mueller 1993) concluded that the **St** and **Y** genomes were not related. Based on molecular markers and gene sequences, Gao et al. (2014) and Hu et al. (2015) supported the previous conclusion that the **St** and **Y** genomes originated from different donors independently. Conversely, Liu et al. (2006) using ITS (internal transcribed spacer) sequence data suggested that the **Y** genome was derived by a gradual differentiation from the **St** genome. Okito et al. (2009) suggested that the **Y** genome in some *Elymus* species shares a progenitor genome (designated **St^Y**) with the **St** genome of *Pseudoroegneria*. Based on six HMW-GS genes, Zhang et al. (2016) reported that the two derived genomes in *Roegneria* were from the **St** genome rather than the **Y**, but have undergone slight differentiation. Based on morphology and chromosome pairing in hybrids between Eurasian and North American *Pseudoroegneria* and *Roegneria* species, Jensen et al. (1995) concluded the North American *P. spicata* (Pursh) Á. Löve, Eurasian *P. strigosa* (M. Bieb.) Á. Löve, *P. geniculata* (Trin.) Á. Löve, *Rogneria elytrigoides* C. Yen et J. L. Yang, and *R. glaberrima* Keng & S.L. Chen share the same basic **St** genome.

Roegneria alashanica Keng is a tetraploid perennial species ($2n = 4x = 28$) with awnless lemmas and glumes length about half the length of the first lemma (Yu et al. 2010). Cai (1997) treated *R. alashanica* in the genus *Roegneria* and reduced *R. elytrigoides* to a taxonomic variety *Roegneria alashanica* var. *elytrigoides* (C. Yen et J. L. Yang) L. B. Cai. Previously, Lu (1994) demonstrated by chromosome pairing in hybrids that *R. elytrigoides* contained two slightly different **St** genomes and renamed it as *Pseudoroegneria elytrigoides* (C. Yen et J. L. Yang) B. R. Lu. Using the random amplified polymorphic DNA (RAPD) described by Wei and Wang (1995), Ding et al. (2005) obtained data suggesting that *R. alashanica* and *R. magnicaespes* (D. F. Cui) L. B. Cai possess the **StStStSt** or **St₁St₁St₂St₂** genomes, like *P. elytrigoides*. Liu et al. (2006) then

confirmed that **StSt** is the genomic constitutions of *R. alashanica* and *P. elytrigioides* based on ITS analyses of *Elymus* L. species.

However, based on genomic *in-situ* hybridization (GISH) Yu et al. (2010) concluded that *R. alashanica* and *R. magnicaespes* contained one **St** genome and one unidentified genome, which was not an **E**, **P**, **H**, or **Y** genome. In their study, *R. grandis* Keng contained **StSt^g** genomes. The **St^g** genome was suggested to be a modified form of the **St** genome and homoeologous with the **Y** genome. Thus, **St^g** might be an intermediate type between the **St** and **Y** genomes. The **St^g** genome would be similar to the **St^Y** proposed by Okito et al. (2009). Contrarily, Zhang et al. (1998) reported that *R. grandis* had **StY** genomes based on chromosome pairing in artificial hybrids. Svitashv et al. (1998) also suggested that *R. grandis* contained **StY** genomes based on the analysis of genome-specific repetitive DNA and RAPD markers. Both Zhang et al (2009) and Lei et al. (2016) concluded that the genome of *R. grandis* should be designated as **St^gY**. But, Zhang et al. (2009) demonstrated that *R. alashanica* had one set of modified **St** genome that was closely related to the **E^c** genome, while the other set of genome was closely related to the **P** genome. Whereas, Lei et al (2016) showed that *R. alashanica* had one copy of disrupted meiotic cDNA (DMC1) sequences coming from **St** genome and another from **Y**, indicating **StY** is the genome constitution of *R. alashanica*.

Thus, the genome constitution of *R. alashanica* remains unknown and requires clarification. In the present study, we used a **Y**-genome specific sequence tagged site (STS) marker that was converted from a RAPD marker (Okito et al. 2009) to test whether *R. alashanica* contains the **Y** genome. Results from this test would determine if *R. alashanica* needs to be transferred to the genus *Pseudoroegneria*.

Materials and Methods

Number of accessions, plants per accession, and genome make-up of *E. longearistatus* (Boiss) Tzvelev, *R. alashanica*, *P. strigosa* (M. Bieb.) Á. Löve, *P. libanotica* (Hackel) D.R. Dewey, and *P. spicata* (Pursh) Á. Löve used to assess the presence of Y-genome specific STS marker, B14(F+R)₂₆₉ were listed in Table 1. Two collections, PI 401278 and 401282, of *E. longearistatus* used in this study were collected as *A. longearistatum* between Gachsar and Shemshak about 50 km north of Tehran, Iran in 1972, with PI 401282 exhibiting green, taller reproductive culms 65 cm compared to purple culms 50 cm tall in PI 401278. The *R. alashanica* accession Z-1081 was donated by Prof. Dong Yu-Shen, Director, Crop Germplasm Resources Institute, Chinese Academy of Agricultural Sciences, Beijing, China in 1990. This accession was previously used by Zhang et al. (2016). *Pseudoroegneria strigosa* (W6 14033) originated from Tioanchi, Tianshan, Fukang, Xinjiang, China where it was found growing in cliff crevices. *Pseudoroegneria libanotica* accession PI 228392 originated from Iran, whereas PI 401326 was collected 140 km southeast of Tabriz toward Zanjan on steep rocky slopes in 1972. *Pseudoroegneria spicata* collections PI 236668 and 236682 originated from Canada, while PI 232134 originated from Wyoming, USA.

DNA extraction, PCR amplification, and visualization of electrophoresis gels for PCR products followed procedures described by Okito et al. (2009), except that individual plants were used instead of bulked DNA samples. Approximately a 100 mg of fresh leaf tissue was collected from each seedling for DNA extraction using the cetyltrimethyl-ammonium bromide (CTAB) method (Rogers and Bendich, 1988). Genomic DNA was quantified and normalized to 40 ng/μl. The PCR reaction mixture was composed of 10 μl Buffer 10X, 0.2 mM of each deoxynucleotide, 25 mM MgCl₂, 10 mM dNTP, 1U Taq polymerase, 4 μl of each primer, and 20 or 40 ng template DNA in

a final volume of 25 μ l. Template DNA was normalized according to ploidy levels so that each genome was approximately 20 ng, i.e., 20 ng for **St**-genome diploids and 40 ng for **StY**- or **StSt**-genome tetraploids. PCR was performed using a Applied BioSystem 9700 thermocycler programmed to run 95°C for 2.5 min followed by 30 cycles of a denaturing step of 95°C for 2.5 min, an annealing step of 55°C for the **Y** genome marker, and an extension step of 72°C for 5 min. The PCR product was mixed with 10X loading dye solution by using 7 μ l of the total 25 μ l PCR reaction product and 3 μ l of loading dye, and then analyzed by electrophoresis on a 2% agarose gel. DNA profiles were recorded using an UVP 2UV Transilluminator imaging system. The size of the fragments was estimated using 100 bp ladders.

Results and Discussion

All 11 and 18 plants of *E. longearistatus* accession PI 401278 and PI 401282, respectively, produced an intense band of the **Y**-genome specific STS marker, B14(F+R)₂₆₉ (Table 1). In addition to the most intense band of 269bp, there were bands around 600bp and 900bp (Fig. 1A, lanes 1 to 11), suggesting that the **Y**-genome specific sequence is present as tandem repeats. Okito et al. (2009) estimated that the 269bp sequence was present in *E. longearistatus* in approximately 2,900 copies. Whereas, the relative copy number of B14(F+R)₂₆₉ in the whole **Y** genome of *E. longearistatus* (**StY**) and *E. rectisetus* (**StWY**) was 58 and 17, while that in a pair of 1**Y** and an unidentified **Y**-genome chromosome of *E. rectisetus* was 1 and 2, respectively (Dou et al. 2012). *Elymus longearistatus* has about 3.6 fold more of B14(F+R)₂₆₉ repeats than in *E. rectisetus*; and 1**Y** chromosome of *E. rectisetus* has approximately 50 copies of the repetitive sequence. These results indicated that **Y**-genome chromosomes varied in copy number of the B14(F+R)₂₆₉ sequence both within and among species containing the **Y** genome.

Only 6 out of 10 plants of *R. alashanica* amplified a relatively faint band of the **Y**-genome specific STS marker (Fig. 1A, lanes 12 to 21). The intensity of this band in *R. alashanica* was similar to that in one plant of *P. libanotica* PI 401326 (Fig. 1B, lane 17). The **Y**-genome specific STS marker in PI 401326 had been previously estimated to possess one copy of B14(F+R)₂₆₉ in the whole **St** genome of *P. libanotica* (Okito et al. 2009). The **St** genome having a low copy number of the **Y**-genome specific STS marker was designated as **St^Y** (Okito et al. 2009). Therefore, we infer that *R. alashanica* has one **St^Y** genome.

The presence of **Y**-genome specific STS marker in *P. libanotica* PI 401326 (Okito et al. 2009) is verified herein (Table 1). However, presence of **Y**-genome specific STS marker in *P. spicata* PI 232134 is not verified. It is possible that one *P. spicata* sample in Okito's study was mislabeled due to the large number of plant materials studied. Nevertheless, variability in the existence of **Y**-genome specific sequence within an accession of a species has now been detected in both diploid (*P. libanotica*) and tetraploid (*R. alashanica*) species having the **St^Y** genome (Table 1). The variability suggests changes in DNA sequences, i.e., diminution and amplification, occurred in the plant populations examined. Thus, **St^Y** could have evolved either to **St** in *P. spicata* by diminution or total loss of the **Y**-genome specific sequence or to **Y** of *Roegneria* by amplification and dispersion of this repeated DNA sequence.

The **St^Y** genome proposed in Okito et al. (2009) and the present study appears to be different from the **St^g** in *R. grandis* (Zhang et al. 2009; Yu et al. 2010; Lei et al. 2016). The **St^g** genome was a differentiated form of the **St** genome in *Pseudoroegneria* and is homoeologous with the **Y** genome in *Roegneria* (Zhang et al. 2009; Yu et al. 2010). If *R. alashanica* possesses the **StSt^Y** genome as identified in this study, the **St^Y** could be hybridized by neither **St** nor **Y** genomic DNA probe in the GISH study of Yu et al. (2010). On the other hand, the **St^g** genome of *R. grandis* could

be hybridized by **St** genomic DNA probe (Yu et al. 2010). The difference between **St^Y** and **St^g** as well as why **St^Y** could not be hybridized by the **St** probe in GISH are not known, but could be related to the degree and nature of genome differentiation. The genome relationships among **St**, **St^g**, **St^Y**, and **Y** are hypothesized in Fig. 2. **St^Y** is remotely related to either **St** or **Y** while **St^g** is more closely related to both **St** and **Y**, so that **St^Y** could not be hybridized by **St** or **St^Y** GISH probe (Yu et al. 2010). These genome relationships could also explain the chromosome pairing pattern $20.40 \text{ I} + 3.69 \text{ II} + 0.09 \text{ III} + 0.04 \text{ IV}$ in the hybrid of *R. grandis* (**St^gY**) and *R. alashanica* (**StSt^Y**) observed by Zhang et al. (2002). The observed bivalents were mostly formed between the **St** and **St^g** genomes, the trivalents detected were among the three versions of **St**, and the quadrivalents were among the four genomes in the hybrid.

Based on morphological and cytological evidence, tetraploid species *R. alashanica* and *R. elytrigioides* are closely related (Lu 1994; Cai 1997). Lu (1994) determined the genomic constitutions of *R. elytrigioides* as **StSt**, and transferred it to *Pseudoroegneria*. Zhang et al. (2002) indicated that *R. alashanica* contains one **St** genome and an unknown genome. Considering morphology, karyotypes, genome-specific RAPD markers, and cytogenetic studies, Ding et al. (2005) suggested that *R. alashanica* had the same genome constitution of *P. elytrigioide*. Liu et al. (2006) assumed the **StSt** as the genomic constitutions of *R. alashanica* for phylogenetic analysis of *Elymus* based on ITS data. A study of nuclear rDNA ITS sequences by Yu et al. (2008) found that *P. alashanica* and *P. elytrigioides* were grouped together because of the shared **StSt** genome constitution. Based on analysis of a gene encoding plastid acetyl-CoA carboxylase (*Acc1*) in various tetraploid *Roegneria* and *Pseudoroegneria*, Zhang et al. (2009) concluded that *R. alashanica* contained one set of modified **St** genome which was closely related to the **E^e** genome of *Lophopyrum elongatum* (Host) Á. Löve, and the other set of genome was closely related to the

P genome of *Agropyron*. They also indicated that the **Y** genome possibly originated from the **St** genome, and was a sister genome to the **St**, **E^c**, **E^b** of *Thinopyrum bessarabicum* (Savul. & Rayss) Á . Löve, and the **W** genome of *Australopyrum*. Most recently, Zhang et al. (2016) found that the two HMW-GS genes of *R. alashanica* were from two **St** genomes undergoing slight differentiation. In contrast, only one study concluded that *R. alashanica* had the genome constitution **StY** (Lei et al. 2016).

Correct classification of species will benefit all biological researchers in their planning of experiments; for example, plant breeders design hybridization combinations to transfer desirable traits from one species to the target crop. Based on the **Y**-genome specific STS marker analysis conducted herein, *R. alashanica* does not contain the **Y** genome. The accession of *R. alashanica* used by Zhang et al. (2016) was Z1081, the same used in our study. Although different research methods were used, results from the present study led to the same conclusion made by Zhang et al. (2016), that is that *R. alashanica* contains two **St** genomes having undergone slight genome differentiation. The genome designation for *R. alashanica* should be **StStSt^YSt^Y** and, thus, it is justifiable to reclassify this species to the genus *Pseudoroegneria* with the following new name combination *Pseudoroegneria alashanica* (Keng) R. R.-C. Wang and K. B. Jensen, which had the following synonyms:

Roegneria alashanica Keng, 1957, Clav. Gen. & Sp. Gram. Sin. 67: 185 (nom.nud.)

Roegneria alashanica Keng, 1959, Fl. Ill. P1. Prim. Sin. Gram. 375 (Chinese description)

Roegneria alashanica Keng, 1963, Acta Nanking Univ. (Biol.): 73 (Latin description)

Roegneria alashanica Keng et S. L. Chen, 1963, Acta Nanking Univ. (Biol) **3**: 1-92.

Elymus alashanicus (Keng) S. L. Chen, 1987, Bull. Nanjing Bot. Gard. 8

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Table 1. Materials used in this study and results of PCR amplification with primers for the Y-genome specific STS marker, B14(F+R)₂₆₉.

Species	Accession	Genome	No. plants	No. plants having B14(F+R) ₂₆₉ marker as	
				intense band	faint band
<i>Elymus longearistatus</i>	PI 401278	StY	11	11	0
	PI 401282	StY	19	19	0
<i>Pseudoroegneria alashanica</i>	Z-1081	St₋	10	0	6
<i>P. strigosa</i>	W6 14033	St	4	0	0
<i>P. libanotica</i>	PI 228392	St	16	0	0
	PI 401326	St^Y	8	0	1
<i>P. spicata</i>	PI 236668	St	5	0	0
	PI 236682	St	13	0	0
	PI 232134	St	11	0	0

Figure legend

Fig. 1. Gel electrophoresis of polymerase chain reaction products amplified by B14 forward and reverse primers showing the **Y**-genome specific STS marker of 269 bp in length (arrowed). (A) The marker was present as an intense band in all plants of *Roegneria longearistatus* accession PI 401278 (lanes 1 to 11) and as a faint band in six (lanes 12, 13, 14, 15, 17, and 20) out of 10 plants of *Pseudoroegneria alashanica* accession Z-1081 (lanes 12 to 21). (B) The marker was present as a faint band only in one plant (lane 17) of 8 *Pseudoroegneria libanotica* PI 401326 plants (lanes 12 to 19), but absent in *P. libanotica* PI 228392 (lanes 1 to 11) and *P. spicata* PI 236668 (lanes 20 to 24). M contains the 100 bp DNA ladders as size markers, of which the two intense bands (1,000 bp and 500 bp) are indicated.

Fig. 2. Hypothetical genome relationships among **St**, **St^g**, **St^Y**, and **Y**. **St^g** is intermediate between **St** and **Y**.

58x38mm (300 x 300 DPI)

23x25mm (300 x 300 DPI)