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Origins Of The Y Genome In Elymus

Pungu Okito
Utah State University

ORIGINS OF THE Y GENOME IN *ELYMUS*

by

Pungu Okito

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Approved:

_____	_____
Dr. Yanju Wu Major Professor	Dr. Richard R.-C. Wang Committee Member/Supervisor
_____	_____
Dr. David Hole Committee Member	Dr. Byron R. Burnham Dean of Graduate Studies

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ABSTRACT

Origins of the Y Genome in *Elymus*

by

Pungu Okito, Master of Science

Utah State University, 2008

Major professor: Dr. Yanju Wu
Department: Plants, Soils, and Climate

The Triticeae tribe DUMORTER in the grass family (Poaceae) includes the most important cereal crops such as wheat, barley, and rye. They are also economically important forage grasses. *Elymus* is the largest and most complex genus with approximately 150 species occurring worldwide. Asia is an important centre for the origin and diversity of perennial species in the Triticeae tribe, and more than half of the *Elymus* are known to occur in the Asia. Cytologically, *Elymus* species have a genomic formula of **StH**, **StP, StY**, **StStY**, **StHY**, **StPY,** and **StWY**. About 40% of *Elymus* species are still unknown for the genomic constitution and some have questionable genomic combination. However, the origin of the **Y** genome is unknown. In order to identify the origin of the **Y** genome, 212 accessions of *Elymus*, *Pseudoroegneria*, and *Hordeum* species were tested using a **Y** genome specific Sequence Tagged Site (STS) marker. We obtained evidence supporting the hypothesis that the **Y** genome in some *Elymus* species shared a progenitor genome with the **St** genome. Our study suggested that *Pseudoroegneria*

spicata (PI 232134), *P. ferganensis* (T-219), and *P. libanotica* (PI401326) are the donors of the **Y** genome in the *Elymus* species. The DNA sequences of the **Y**-genome marker in these three *Pseudoroegneria* species are more similar to those obtained from *Elymus* species having the **Y** genome than those from *Hordeum marinum* and *H. murinum*, making the **Xa** and **Xu** genomes less likely the donors of **Y** genome.

(76 pages)

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Pungu Okito

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ABBREVIATIONS

CTAB	Cyltrimethyl-ammonium bromide
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphates
EDTA	Ethylenediaminetetra-acetic acid
$MgCl_2$	Chloride magnesium
PCR	Polymerase Chain Reaction
X-gal	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside
STS	Sequence Tagged Site
RAPD	Random Amplified Polymorphic DNA
UV	Ultra violet

INTRODUCTION

Elymus

Elymus L. (Triticeae, Poaceae) is composed of approximately 150 perennial species; thus, it is the largest and most morphologically diverse genus in the Triticeae. *Elymus* plants are generally green, lax-leafed, caespitose, self-pollinating grasses. Asia has the largest number of *Elymus* species, many of which have never been thoroughly studied. Hence the genome constitution of many *Elymus* is not known (Barkworth, http://herbarium.usu.edu/Triticeae/genomes.htm). The genus *Elymus* is a complex group of polyploids (more than 2x chromosomes) originated through a typical alloploidy (multiple copies of different genomes) process (Liu et al. 2006). It has been reported by Stebbins and Ayala (1985) that more than 80% of the Gramineae family have undergone polyploidization during their speciation. The occurrence of polyploidy in *Elymus* may contribute to the facts that they are more resistant to cold, heat, and drought, and are better adapted to new environmental conditions than their diploid progenitors. According to Stebbins and Vaarma (1954) the ancestors of *Pseudoroegneria spicata* and *Hordeum* migrated from Asia to North America, hybridized and gave rise to some of the North America polyploids, and then later migrated to South America. Polyploid species of *Hordeum stenostachys* are more widespread than diploid. Therefore, the genus *Elymus* is a model for studying morphological variability, phenotypical plasticity, and natural hybridization. The genus also provides excellent plant materials for cytogenetics, molecular genetics, and phylogeny investigations (Diaz et al. 1999b) .

The designation of individual basic genomes has been a problem in Triticeae since the tribe has many poorly studied species (Wang and Jensen 1994). Among *Elymus* species, some still have questionable genomic constitutions and forty percent still have unknown genomes (Assadi and Runemark 1995; Svitashev et al. 1996). The relation between the genomes in Triticeae perennial species is not fully established.

There has been confusion between some genome symbols used by scientists and researchers who were studying wheat and related species in the Triticeae tribe. Several researchers have proposed a new system of designation which is less confusing and easy to understand (Wang and Jensen 1994; Wang et al. 1995) To design a genome they decided: (1) to use bold face, (2) the unknown genome should be designated with the letter **X** followed by a lower-case letter, for example, **Xu** for *Hordeum murinum* and **Xa** for *Hordeum marinum*, and (3) **Y** genome is retained to design an unidentified diploid species that contributed a genome to some species of *Elymus*.

In Triticeae, the basic haplome (individual with **n** chromosomes) is **x = 7** (Wang et al. 1995). The number of chromosomes in *Elymus* is between **2n = 4x = 28 to 2n = 8x = 56** (Jensen and Salomon 1995; Jensen and Asay 1996; Ellneskog-Staam et al. 2007).

Lu and Salomon (1993a) reported that Asia is an important center of origin and diversity for *Elymus*. Asia has the highest number of *Elymus* species, differing in polyploidy levels, morphological characters, and genomic combinations. They distributed the *Elymus* species according to their genomic constitution and geographic distribution in Asia (Figs. 1-4).

Geographically, *Elymus* species from the Tibetan plateau of China have a higher genetic variation than those found in the Inner Mongolian plateau. In contrast, *E. glaucus*

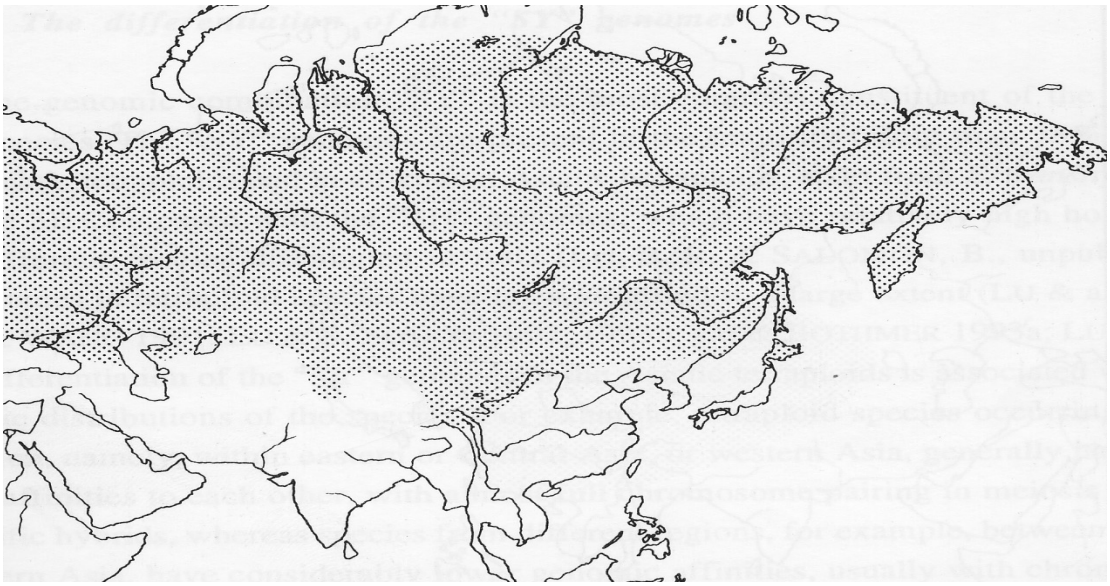

Fig. 1. A general distribution map of StH genome *Elymus* species in Asia. Excerpted from Lu (1993a)

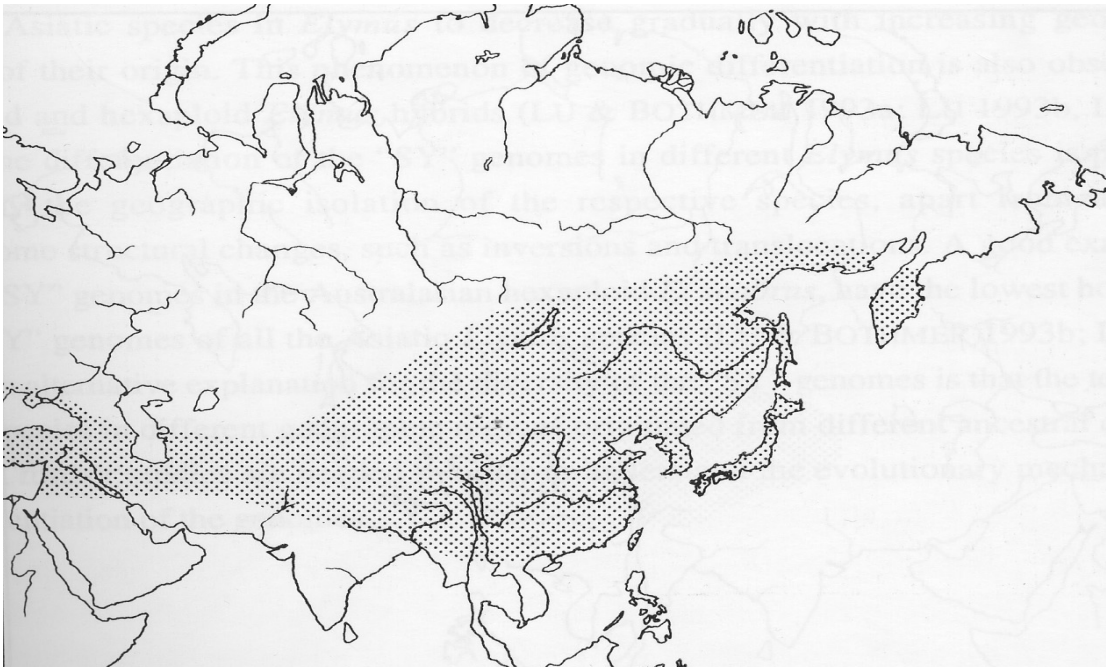

Fig. 2. A general distribution of StY and StStY genome of *Elymus* species in Asia. Excerpted from Lu (1993a)

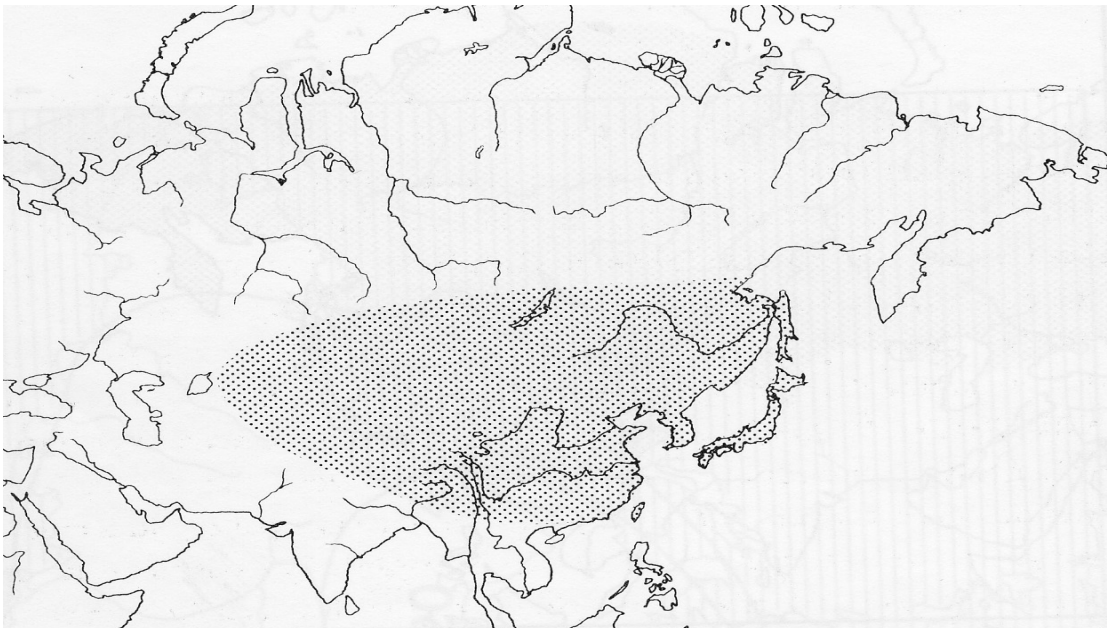

Fig. 3. A general distribution of StYH genome *Elymus* species in Asia. Excerpted from Lu (1993a

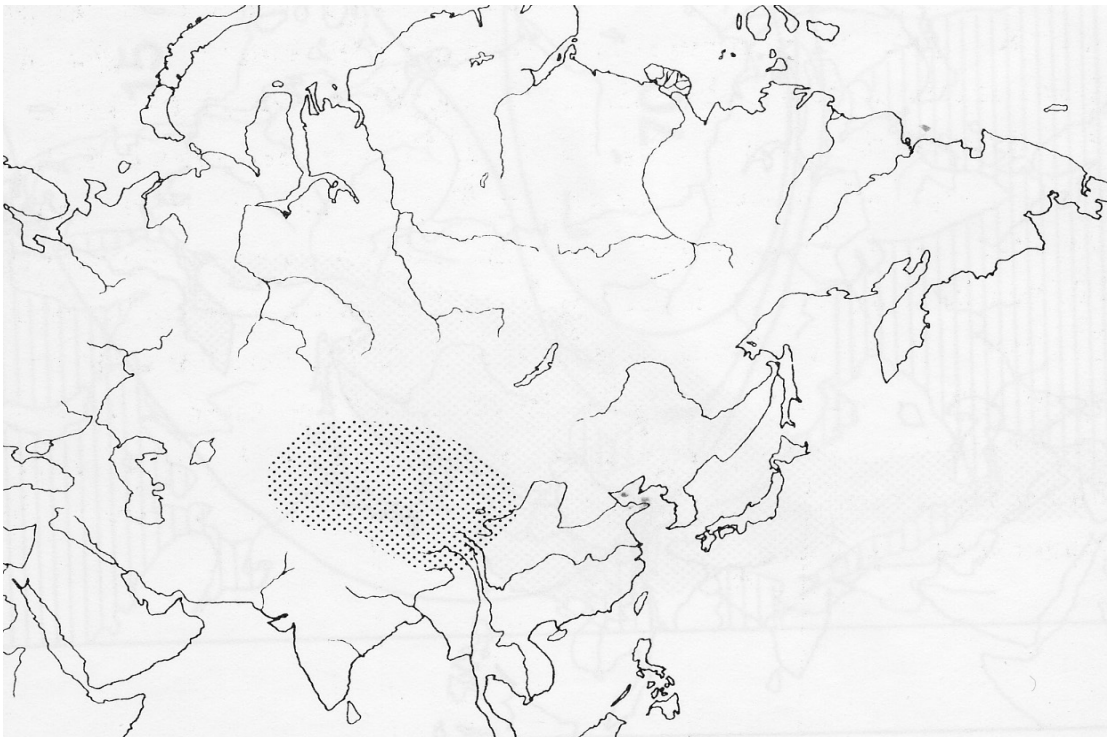

Fig. 4. A general distribution of StYP genome *Elymus* species in Asia. Excerpted from Lu (1993a)

and *E. longiaristatus* have the lowest genetic diversity values (Yan et al. 2007). Lu and Salomon (1993b) reported that the degree of genomic affinity between *Elymus* species is and *E. longiaristatus* have the lowest genetic diversity values (Yan et al. 2007). Lu and Salomon (1993b) reported that the degree of genomic affinity between *Elymus* species is to a far larger degree associated with their geographic distribution than their morphological similarity. The same results have been found with the **StY** genomes in *Elymus nutans*, which have the highest homology with the corresponding genomes in tetraploids such as *Elymus pseudonutans*, which occurs in the same region as *Elymus nutans*. Lu (1993a) also found that **StY** in the tetraploid *E. caucasius* from West Asia and the **StYW** genomes in hexaploid *E. scabrus* from Australia have the lowest homology with **StY** genomes in *E. nutans*. Lu (1993a) reported that the genomic affinity of *Elymus* and *Pseudoroegneria* gradually decreases with increasing geographic distance between the species.

Cytogenetic techniques have been used extensively in studies of *Elymus* species to clarify their evolutionary origins and genetic relationships. Dewey (1980) was the first to describe the genomic constitution of Central Asian hexaploid (2n = 42). Based on the system of classification and definition of the genome, it has been suggested that the genome combinations for *Elymus* species include: **StH**, **StY**, **StP**, **StStH**, **StHY**, **StPY**, and **StWY** (Dewey 1984; Baum et al. 1991; Wang 1992; Wang et al. 1995; Larson et al. 2003)

Chromosome pairing in hybrids between *Elymus* and *Pseudoroegneria* species demonstrated that all the *Elymus* species share a common **St** genome originated from the genus *Pseudoroegneria* (Dewey 1980; Wang 1992). The **H** genome originated from

Hordeum; **P** from *Agropyrom* and **W** from *Australopyron*. The letters **X** and **Y** have been assigned by various authors to denote the unidentified genomes in Triticeae species (Wang et al. 1995). The **Y** genome is found in many polyploidy species of *Elymus* from Central Asia, eastern to Japan (Dewey 1980). About 30 **StY** genome *Elymus* species are found restrictedly in temperate Asia (Liu et al. 2006). Torabinejad and Mueller (1993a) experimentally demonstrated that *E. rectisetus* and *E. scabrus*, which are endemic to New Zealand and Australia, shared the three genomes, **St, Y,** and **W**. The **W**-genome in the two genera was reported to have fairly high homology, but the **W** genome has very low homology with any other genomes in *Elymus* (Torabinejad and Mueller 1993a) .

The chromosome pairing studies suggested that all the North American species of *Elymus* are **StStHH** allotetraploids (Mason-Gamer et al. 2002). *Elymus canadensis* is an example of tetraploid (2n = 2x = 28) with a basic genome combination of **StStHH**.

Analysis of chromosome-pairing confirmed the presence of the **St** and **Y** genomes in *E. borianus* and suggested that the genomic formula of this species should be **StYX**, with **X** and **Y** symbolizing the unknown genomes (Svitashev et al. 1998).

From cytological analysis of artificial hybrids among **StY** species, evidence suggested that the degree of chromosome pairing in the hybrids gradually decrease with increase in geographical distance between the localities of their parental species (Lu and Salomon 1993b; Liu et al. 2006).

In addition to being the largest genus in the Triticeae, *Elymus* is also the most widely distributed (Dewey 1984; Jensen and Asay 1996). *Elymus* occur from the Arctic, to temperate and subtropical regions, and they are most abundant in Asia and North America. The genus extends from North America into Europe, South America, and

Australia (Barkworth and Dewey 1985; Wang 1992; Jensen and Asay 1996; Lewis et al. 1996). Approximately 80 of the known *Elymus* species originated in Asia. North America has the second largest number of endemic *Elymus*, approximately 50 species (McMillan and Sun 2004). *Elymus* inhabits various ecological niches including grassland, semi desert, mountain slopes, valleys, and forest regions (McMillan and Sun 2004). *Elymus* species have been examined in relation to structural and environmental variables, including vegetative structure, geographic and topographic position, soil physical characteristics and macronutrient levels, temperature, and precipitation, as well as factors of disturbance, including fire, light, grazing, and air pollution.

Abiotic factors such as waterlogging and salinity are not limiting factors for the distribution of *Elymus* species (Bockelmann and Neuhaus 1999). However, waterlogging can cause severe yield reduction in wheat crops throughout the world (McDonald et al. 2001).

Elymus are characterized as nitrophilous because of the high concentration of annual atmospheric nitrogen input of 16 kg ha^{-1} y^{-1} (Bockelmann and Neuhaus 1999). Distribution of *Elymus* can also be affected by the availability of additional atmospheric nitrogen. *Elymus* species are also regarded as halophytic plants and are often found growing in salinity affected soils or where they are exposed to salt spray. These habitats include saline semi-deserts, mangrove swamps, marshes, sloughs and seashores.

The review of the various species of *Elymus* might best be presented according to their genomic constitutions and geographic distribution.

StH genome species. It is generally known that all American *Elymus* species contain **St** coming from *Pseudoroegneria* species and **H** from *Hordeum bogdanii*

Wilensky (Dewey 1980). These include *Elymus sibiricus, E. caninus, E. alaskanus*, *E. trachycaulus, E. elymoides, E. canadensis, E. lanceolatus, and E. glaucus.*

Elymus caninus (L.) L (2n = 4x = 28) grows from Iceland and the British Isles to Siberia and from the Sun-arctics to the Mediterranean (Diaz et al. 1999a; Sun et al. 1999). It inhabits forests with shaded canopies (Jensen 2005; Mizianty 2005). Only two species of *Elymus* are found in Iceland, *E. caninus* L. and *E. alaskanus* (Scribn. & Merr.) Löve. *E. caninus* tends to occur in birch shrubs on rather wet soils. These two species are currently disappearing, probably due to grazing (Ørgaard and Anamthawat-Jónsson 2001). *E. caninus* is widespread, both towards the east and the west of Iceland (Ørgaard and Anamthawat-Jónsson 2001). *Elymus trachycaulus* (Link) Gould ex Shiners (**StH**) extends from Eurasia through Alaska to Newfoundland, and to Mexico along the Rocky Mountains. Usually it grows in the open forests and along roadsides (Sun et al. 2006). The *E. trachycaulus* complex probably originated from multiple North American populations. There is a high genetic diversity found among species of the *E. trachycaulus* complex of North America (Sun et al. 2006). *Elymus trachycaulus* (**StH**) complex is known to form stable hybrids but the genetic basis of the characters used to distinguish its taxonomy is not known (Sun et al. 2006).

Elymus elymoides, having the genome combination **StH** (Stebbins and Vaarma 1954), has been reported as the parent in natural hybrids between tetraploid *Elymus* species (Jensen et al. 1999).

Elymus canadensis L., common name Canada wildrye, is a **StH** tetraploid (Dewey 1975) originated from North America and is widely distributed throughout United States and northwestern Canada (Park and Walton 1990a). *E. canadensis* has been hybridized

with other Triticeae grasses and cereals to develop new germplasm better adapted to environmental and disease stresses and to study genome relationships (Dewey 1984). Park and Walton (1989) reported that the nature of the **St** and **H** genomes and genetic variability in *E. canadensis* were not extensively studied.

Elymus lanceolatus (Scribn. & Smith) Gould is a **StH** tetraploid (Dewey 1984; Jensen and Wang 1991, 1997); with the common name thickspike wheatgrass. It is a long lived native of northern Great Plains and Intermountain regions of North America (Humphrey and Pyke 1997). It is found on the lower dry plains in the central Idaho and up to 10,000 ft in the Wasatch Mountains (Jensen et al. 2001). It occurs in sagebrush steppe, where patchiness of soil resources is important (Jackson and Caldwell 1993).

Elymus glaucus, blue wildrye, is a native species of North America found on the burned-over forest lands. It is distributed in the western U.S., most commonly found in forested areas from western sea coast to the high elevation in the Rocky Mountain (Jensen et al. 2001).

StY genome species. *Elymus longearistatus* (Boiss.) Tzvelev, **StY** (Jensen and Wang 1991), is found on the stony slopes and rocks in the middle and upper mountain belts of eastern Asia, and western Pamir of the former USSR, Turkmainia, and Iran (Tzvelev 1976). Geographically, they are located around the Caspian Sea.

Elymus gmelinii was originally described as *Tritcum caninum* L. var. *gmilinii* by Carl Friedrich Von Ledebour in 1829. Its habitats include meadows, forest glades and sparse forest up to the middle mountain belt of Central Asia, including portions of Western Siberia (Jensen and Hatch 1989). It is also found in the region of Japan (Tzvelev 1976; Lu and Salomon 1992).

StPY genome species. *Elymus alatavicus* (Drob.) A. Love (**StPY)** are natives of Central Asia. They grow on stony slopes and screes in the central and upper mountain belts of the Tien-Shan and Pamir-Altai mountain ranges of Central Asia (Tzvelev 1976;(Tzvelev 1976; Jensen 1990). *Elymus kengii* (Keng) Tzvelev (**StPY**) is a grass from west central China (Jensen 1990). The presence of **P** genome in the *E. kengii* demonstrated that *Agropyrum* species have been involved in the evolution of polyploids within *Elymus* (Jensen 1990).

StHY genome species. *Elymus drobovii* possesses the **St**, **H**, and **Y** genomes (Dewey 1980; Dewey 1984; Jensen 1990; Wang et al. 1995), reported that *E. drobovii* was not recognized as a valid species until 1932 when Nevski described it from a specimen collected in 1920 in the vicinity of the Tashkent in the former USSR.

Elymus nutans Griseb is a hexaploid species (2n = 6x = 42) with genomic formula of **StHY** (Lu 1993b). It has been described in the western Himalaya, but widely distributed in Asia and grows in grassland, among the bushes, along the river banks, on the mountain slopes and in the swales, at altitudes from 1000 m up to 5000 m (Lu 1993b). It is a well-known species in the *Elymus* genus and covers a large area in China, India, Pakistan, Mongolia and Russia, and it occupies various ecological habitats (Lu 1993b). *Elymus nutans* has the highest genomic homology among hexaploid *Elymus*.

StYW genome species. *Elymus rectisetus* (Nees in Lehm.) A. Love & Connor, hexaploid, has the genome combination **StStYYWW**. *Elymus rectisetus* and *E. scabrus* are endemic to Australia and New Zealand and both share the same three genomes (Torabinejad and Mueller 1993b; Redinbaugh et al. 2000).

Economic importance. *Elymus* is an excellent model material for research on genetic diversity, cytogenetics, molecular genetics, phylogeny and speciation (Diaz et al. 1999b). The genus comprises both wheatgrasses and wildryes. Only two wheatgrasses in *Elymus*, *E. lanceolatus* (thickspike wheatgrass) and *E. trachycaulus* (Link) (slender wheatgrass) are agronomically important. They are known to be drought resistant (Dewey 1984; Jensen 1996; Jensen and Asay 1996), and are used for revegetation, soil stability, and erosion control (Knapp and Rice 1996). *Elymus trachycaulus* (Link) is used for restoration of disturbed lands (Brown et al. 1978; Jensen et al. 2001). It contains genes used to improve barley and wheat resistance to Russian wheat aphid (Aung 1991). It is a bunchgrass and was first native grass to be used in the revegetation program in the western US and Canada. *Elymus trachycaucus,* known as slender wheatgrass is a short-lived native bunchgrass with good seedling vigor and moderate palatability (Jensen et al. 2001). Due to its rapid seed germination and establishment, moderate salt tolerance and compatibility with other species, it is a valuable component in erosion-control and mine land reclamation seed mixes. Slender wheatgrass is used as a cover or nurse crop during establishment of longer lived species (Jensen et al. 2001).

Because of their excellent forage quality, rich in protein and lysine contents, *Elymus* genus are extensively used for grazing in some regions (Dewey 1984; Liu et al. 1994; Sun 2002; Marley et al. 2007a; Marley et al. 2007b). Marley (2007a) suggested incorporating alternative forage in the lamb diet to increase live weight and dry matter.

In Denmark and the Netherlands, *Elymus arthicus* have been used for grazing cows and sheep. However, grazing also leads to edaphic factors such as soil compaction, high denitrification (nitrogen reduction) and lower mineralization (decomposition of

organic matter). *Elymus nutans* is among the *Elymus* species which encompasses many economically important forage grasses (*E. sibiricus* L., *E. trachycaulus* (LINK) Gouldex SHINN., and *E. canadensis* L.). Siberian wildrye (*Elymus sibiricus* L.), is a native Alaskan standard forage cultivar in common use in Alaska. *Elymus alaskanus* (Scribn.and Merr.) Love s.1. is mostly used for forage in Alaska. *Elymus elymoides*, bottlebrush squirreltail, provides energy for grazing animals in winter and greens up early in spring. It becomes unpalatable at maturity. It is used as soil-covering plant for erosion control and as successional species in rangeland plantings (Jensen et al. 2001). *Elymus canadensis* has been used for forage and hybridization with other Triticeae to produce a better germplam (Park and Walton 1990b). It is a robust bunchgrass, well adapted to the cold winters and the north. However, theses species have the lowest palatability and poor forage quality (Anng and Walton 1989).

Elymus genus has a number of valuable agronomic traits (such as disease resistance and stress tolerance, for example. *Elymus athericus* is widely adapted and it is used for plant improvement of cereal crops such as wheat, barley, and rye (Crane and Carman 1987; Dong et al. 1992; Diaz et al. 1999b, a; Sun 2002; Yen et al. 2005; Yan et al. 2007). Geneticists and plant breeders are introducing genes from wild Triticeae species into cultivated species such as wheat particularly for disease resistance (Hoisington et al. 1999).

Pseudoroegneria

Pseudoroegneria (Löve, 1984) consists about 15 species (diploids and tetraploids) based on the **St** genome. *Pseudoroegneria* grasses are perennial. Dewey (1984) stated

that all the species of *Pseudoroegneria* were included in the *Agroporym* and *Elytrigia* before they were transferred to *Pseudoroegneria* by Löve (1984).

Genomic constitution and distribution. *Pseudoroegneria* species are supposed to contain only the **St**-genome based on Löve's genomic system of classification (Löve 1984). However, both artificial and natural hybrids of the **StP** genome constitution have been reported (Wang et al. 1985; Assadi and Runemark 1995). For example, *Pseudoroegneria tauri* is a true allotetraploid having the **StP** genome formula (Wang et al. 1986; Jensen and Wang 1991). Wang et al. (1986) reported that there is a natural hybrid between an Asian *Pseudoroegneria* species and crested wheatgrass, which has been identified and named *Pseudoroegneria deweyi.* These **StP** species were renamed by Yen et al. (2005) as *Douglasdeweya wangii* and *D. deweyi*, respectively. Some 4x *P. spicata* behaved like allotetraploids during their meiosis.

Pseudoroegneria species are distributed in the northern hemisphere (Wang et al. 1985) including North America, Western Europe and Middle East (see *Pseudoroegneria* world distribution map below). *P. spicata* (Pursh) A. Love is the North America species of the genus. Its distribution in the U.S. seems to be limited in the Western region. It extends the south to north edge of Sonoran Desert, Washington, Montana, Colorado, and northern Canada (Jensen et al. 2001). It is known as bluebunch wheatgrass mostly found on the dry mountain slopes and mountain-brush, at middle elevation with sagebrush, ponderosa pine, steppes, rocky slopes, and other shrubs and sometimes at subalpine elevations (Wang et al. 1985). It persists on deep well-drained loamy soils. Bluebunch wheat grass can withstand grazing early in the growing season if the soil water is adequate and grazing animals are removed before the boot stage. *Pseudoroegneria*

spicata forma *spicata*, *P. spicata* forma *inemis* and *P. spicata* forma *pubescens* are found in Oregon, Idaho and Washington State.

Pseudoroegneria libanotica is a morphologically variable species with a distribution from Lebanon, throughout Turkey, Anatolia, Syria and Iran, to Caucasus, and North of Iraq (Assadi and Runemark 1995). *Pseudoroegneria ferganensis* (Neski) A Löve is native to Tadzhitikistan, in Central Asia (see world *Pseudoroegneria* distribution map, Fig. 5). They are mostly found in the dry igneous mountain slopes and limestone ravines at elevations that range from 1600-2000 m. Dewey (1981) reported that *E. ferganensis* grows on stony slopes in the Pamir and Alai mountain ranges of Central Asia (Lu 1993a). It is a perennial, winter hardly and densely tufted with no rhizome when grown under nursery conditions (Dewey 1981).

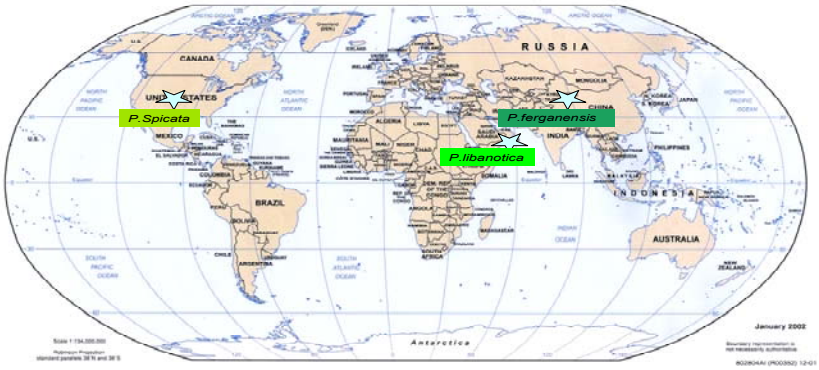

Fig. 5. World distribution map of three *Pseudoroegneria* species (www.Google/image.htm)

Economic importance. *Pseudoroegneria spicata* (bluebunch wheatgrass) and *P. spicata ssp. inermis* (Scribner & Smith) A. Löve (beardless wheatgrass) are used as

forage for livestock and wildlife (Wang et al. 1986), and they are important native grasses of the Palouse Praire. Because of its high nutritional value and palatability, bluebunch wheatgrass is one of the most important and valuable range forage grasses for livestock and wildlife. However, bluebunch wheatgrass regrows rapidly after grazing and is not recommend as a hay crop (Jensen et al. 2001). It can withstand early light grazing before elongation of flowering stems and heavier grazing after seed ripening, but not in between (Jensen et al. 2001). Bluebunch wheatgrass is palatable to all classes of livestock and wildlife. It is also preferred forage for cattle and horses year round but it is considered too coarse in summer. It also is preferred forage for sheep, deer, antelope, sheep and elk in most seasons. In spring the protein levels can be as high as 20% and decreases to about 4% as the plant matures (USDA-NRCS 2003). Bluebunch wheatgrass is very drought resistant, persistent and adapted to stabilization of disturbed soils. It is very compatible with slow-developing native species, such as thickspike wheatgrass (*Elymus lanceolatus*), western wheatgrass (*Pascopyrum smithii*) and needlegrass species (*Achnatherum* spp., *Nassella* spp and *Hesperostipa spp*). This grass can be used in urban areas where irrigation water is limited and to stabilize ditchbanks, dikes and roadsides as a component of a mix (USDA-NRCS 2003). *P. spicata* is highly self-sterile; crosses could be made without emasculation (Asay 1987). Bluebunch wheatgrass is a long-lived drought-tolerant native bunchgrass that begins growth early in spring and regrows with the onset of fall rains (Jensen et al. 2001). Bluebunch wheatgrass prefers coarse-textured soils but will establish and persist on deep well-drained loamy-soils (Jensen et al. 2001).

Hordeum

Hordeum L is a genus of the grass tribe Triticeae with approximately 37 species (Mizianty 2005; Yen et al. 2005; Mizianty 2006), which include the important cultivated barley (*H. vulgare* L.) (Baum and Bailey 1984).

Genomic constitution and distribution. *Hordeum* species have three ploidy levels; diploids (2n = 2x = 14), tetraploids (2n = 4x = 28), and hexaploids (2n = 6x = 42) (Nishikawa et al. 2002), Critesion section of *Hordeum* constitutes a large group with the basic genome **H** (Bothmer et al. 1988). Four genomes are recognized within *Hordeum* L: (1) the **I** genome (often referred to as the **H** genome by plant breeders) shared by *H. vulgare* and *H. bulbosum*, (2) the **Xa** genome of *H. marinum* (2x, 4x), (3) **Xu** genome of *H. murinum* (2x, 4x, 6x) and (4) the **H** genome in mostly the rest of the *Hordeum* species. Löve (1984) made a separation between *Critesion* Rafin from *Hordeum* but some scientists suggest two groups in the genus *Critesion*, they are *H. murinum* L. (= section *Trichosstachys* (Dumortier) A. Löve) and *Hordeum marinum* Huds (=section marina) are different from other *Critesion* species (von Bothmer et al. 1988; Jaaska 1994; Nishikawa et al. 2002) , showed that *Hordeum murinum* is the single species that possess the **Y** genome and consists of three subspecies, (1) *murinum* (4x), (2) *leporinum* (4x and 6x) and (3) *glaucum* (2x). *Hordeum murinum* subsp. *leporinum* (4x) participated in the formation of the hexaploid cytotype as the maternal parent (Nishikawa et al. 2002).

The wild *Hordeum* species are mainly distributed in temperate areas and more concentrated in southern South America and Central Asia (von Bothmer et al. 1988). *Hordeum murinum* and *H. marinum* are annual species found mostly in the Eurasia (von Bothmer et al. 1988). *Hordeum capense* is one species of *Hordeum* that occurs in South

Africa; it seems to be close to the European *H. secalinum,* because of its relation to this group and has been referred as the Eurasian group (von Bothmer and Landström 1988; Taketa et al. 1999). *Hordeum murinum* is native in Europe ranging from England to Sweden and from France to Hungary (Bowden 1962). Members of the *H. murinum* complex are probably the most widespread of all *Hordeum* species (Mizianty 2006). *Hordeum marinum* is also a native of Europe, but occurs in the Western and Southern regions and along the shores of the Mediterranean to Egypt (Bowden 1962). In Poland, Mizianty (2006) reported that *H. murinum* was found in north-eastern but Bothmer et al. (1988) reported that *H. murinum* does not occur in northern-eastern of Poland. According to Mizianty (2006), the geographic differentiation of the Polish populations of *H. murinum* might have resulted from their different routes of migration into Poland, either from Mediterranean area or from Southwest Asia.

Economic importance. The genus *Hordeum* includes the important cereal crop barley (*H. vulgare* L.). This genus has a dual importance economically and ecologically (Sharrow and Motazedian 1987). Approximately 35% of the cultivated land in the world is used to grow the grains such as wheat, barley, oat and rye.

As forage, it is produced in two phases, fall and spring corresponding to its two types of growth (vegetative and reproductive). The vegetative phase usually occurs in fall, and is characterized by 100% of leaf material, representing one-third to one-half of the total dry matter (DM) production. The reproductive phase is in spring which is characterized by rapidly growing plants with an increasing proportion of stem representing one-half to two-third of the total dry matter production.

Hypothesis

No diploid species containing the **Y** genome has been identified, thus the donor species of **Y** genome in *elymus* is still unknown. However, some researchers have hypothesized, based on nuclear ribosomal internal transcribed spacer (ITS) and chloroplast *trnl-f* sequences, that the **St** and **Y** genomes may have originated from the same ancestor genome (Liu et al. 2006). To test this hypothesis, we screened 212 accessions of the *Elymus, Pseudoroegneria* and *Hordeum* species to search for the diploid progenitor donor of the **Y** genome. This work focused primarily on diploid species because allopolyploidy complicates molecular analysis of species relationships.

Objective

The goal of this study is to identify candidate diploid donor of the **Y** genome in the *Elymus* among species and accessions from *Pseudoroegneria*, *Hordeum*, or both.

According to numerous published papers, the late Dr. Dewey made great contributions to the basic knowledge of genomic constitutions in most plants of the Triticeae family, but finding the diploid species that contributed the **Y** genome to these polyploid *Elymus* species had been a life long challenge for him. Attempts to find **Y** genome specific sequence were unsuccessful (Svitashev et al. 1998; Yen et al. 2005).

In this study, some of the *Elymus*, *Pseudoroegneria* and *Hordeum* species were used for searching for the **Y** genome. Those species were selected because of the hypothesis made by scientists that the diploid donor of the **Y** genome has not been found (Dewey 1984; Wang et al. 1986; McMillan and Sun 2004; Xu and Ban 2004; Yen et al.

2005), but the donor of the **Y** genome may be found in accessions of the *Pseudoroegneria* or *Hordeum* species.

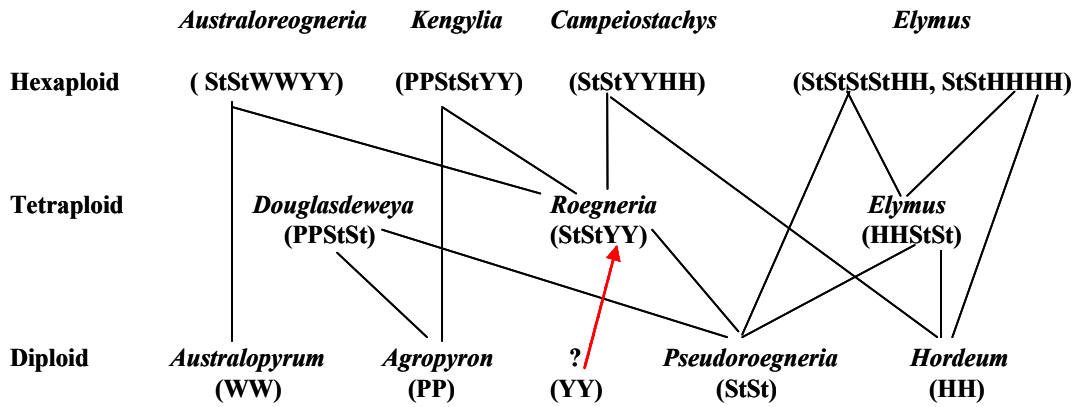

Fig. 6. A diagram of phylogenic relationships among the following genera: *Agropyron*, *Australopyrum*, *Australoreogneria*, *Elymus*, *Hordeum*, *Kengylia*, *Campeiostachys*, *Douglasdeweya*, *Pseudoroegneria* and *Roegneria*. *Campeiostachys* (StStYYHH) originated from *Roegneria* tetraploid (StStYY) and *Pseudoroegneria* (StSt). *Elymus* species have a variety of all the genomes from *Pseudoroegneria* and *Hordeum*. Excerpted from Yen et al. (2005).

In the diagram of phylogenetic relationships among the genera *Agropyron*, *Australopyrum, Australoroegneria, Elymus, Hordeum, Kengyilia, Douglasdeweya, Pseudoroegneria and Roegneria* (Fig. 6), Yen et al. (2005) thought that *Roegneria* (**StStYY)** and *Pseudoroegneria* (**StSt**) may have diploid donors of the **Y** genome, but there was no evidence for such a hypothesis. It was demonstrated in the diagram that species of *Australoroegneria* are hexaploids (**StStWWYY**) originating from diploid parents *Australopyrum* (**WW**), and *Kengylia* (**PPStStYY**) originated from *Roegneria* tetraploid (**StStYY**) and *Agropyron* diploid (**PP**). *Elymus* originated from *Pseudoroegneria* diploid (**StSt**).

MATERIALS AND METHODS

Plant materials

A list of plant materials analyzed together with accession number, genomic constitution, ID number, and ploidy level are found in Tables 1-5.

Table 1. Genomic combination and references of species used in this work (http://www.herbarium.usu.edu/triticeae.htm)

Taxon	Genome	Reference	
Pseudoroegneria	St	Yen et al. 2005	*Pseudoroegneria*
Elymus			
E. alaskanus	StH	Oergaard & Anamthawat-Janson 2001	*Elymus*
E. canadensis	StH	Dewey 1970; Redinbaugh et al. 2000	*Elymus*
E .caninus	StH	Overgaard & Anamthawat-Jonson 2001	*Elymus*
E. elymoides	StH	Mason-Gamer 2001; Larson et al. 2003	*Elymus*
E. glaucus	StH	Dewey 1965; Mason-Gamer 2001, 2002	*Elymus*
E. lanceolatus	StH	Dewey 1967 (as *Agropyron dastachym*); Mason-Gamer 2001; Mason-Gamer et al. 2002	*Elymus*
E. sibiricus	StH	Dewey 1974	*Elymus*
E. trachycaulus	StH	Dewey 1977; Redinbaugh et al. 2000; Mason-Gamer et al. 2002	*Elymus*
E. dahuricus	StHY	Redinbaugh et al. 2000	*Campeiostachys*
E. drobovii	StHY	Dewey 1980	*Campeiostachys*
E. nutans	StHY	Lu 1993	*Campeiostachys*
E. tsukushiensis	StHY	Lu & von Bothmer 1990a; Redinbaugh et al. 2000	*Campeiostachys*
E. caucasicus	StY	Jensen & Wang 1991; Lu & von Bothmer 1993b	*Roegneria*
E. gmelinii	StY	Jensen & Hatch 1989; Lu & Salomon 1992	*Roegneria*
E. longearistatus	StY	Jensen & Wang 1991; Lu & von Bothmer 1993b	*Roegneria*
E. nevskii	StY	Lu & Salomon 1992; Redinbaugh et al. 2000	*Roegneria*
E. alatavicus	StPY	Jensen et al. 1986; Redinbaugh et al. 2000	*Kengylia*
E. batalini	StPY	Jensen et al. 1986; Redinbaugh et al. 2000	*Kengylia*
E. kengii	StPY	Jensen et al. 1990b; Redinbaugh et al. 2000	*Kengylia*
E. rectisetus	StYW	Torabinejad & Muller 1993; Redinbaugh et al. 2000	*Anthosachne (inlitt.2005/10/30)*
Hordeum marinum	Xa	von Bothmer et al. 1986	
Hordeum murinum	Xu	von Bothmer et al. 1987,1988a,1998b	
Hordeum, other	H		

Table 2 *Elymus* species with unknown genomic combinations (http://www.herbarium.usu.edu/triticeae.htm)

Species	Accession #
E. curvatus	PI 531579
E. angustiglumis	PI 531639
E. tianshanicus	T210
E. interruptus	PI 531617
E. laxiflorus	KJ 278
E. scabriglumis	PI 202147
E. kunlunshanicus	CPI 119942
E. praecaespitosus	X- 93019
E. villosus	PI 531703
E. scabrifolius	PI 531544

Table 3. *Elymus* species.

Genus	Species	Accession #	Genome symbols	ID Sample	Ploidy
Agropyron	*cristatum*	PI 499389	**P**	7260	2x
Australopyron	*retrofractum*	PI 531553	**W**	5434	2x
Elymus	*longiaristatus*	PI 401282	**StY**	5992	4x
	arizonicus	PI 531558	**StH**	6749	4x
	canadenisis	PI 531565	**StH**	6750	4x
	caucasicus	PI 531572	**StY**	5026	4x
	batalinii	PI 314462	**StPY**	6745	6x
	kengii	PI 504457	**StPY**	6756	6x
	villifer	KJ-174	**StHY**	6766	6x
	excelsus	W 94039	**StHY**	6767	6x
	rectisetus	PI 533028	**StYW**	6112	6x
	drobovii	PI 314196	**StHY**	6747	6x
	curvatus	PI 531579	?	6751	4x
	angustigglumis	PI 531639	?	6769	4x
	tianshanicus	T 210	?	3248	6x
	tschimganicus	PI 564498	**StStY**	6767	6x
	fibrosus	PI 547320	**StH**	6753	4x
	gmelinii	AJC 266	**StY**	6754	4x
	elgmoides	TAJ 90401	**StH**	2702	4x
	nevski	H-10215	**StY**	6758	4x
	alatavicus	PI 531709	**StPY**	7261	6x
	caninus	PI 547706	**StH**	6746	4x
	interruptus	PI 531617	?	5027	4x
	laxiflorus	KJ 278	?	6757	6x
	scabriglumis	PI 202147	?	6762	6x
	tangutorus	CPI 11975	**StHY**	1280	6x
	tsukushiensis	PI 499624	**StHY**	5464	6x
	nutans	PI 531587	**StHY**	6759	6x

glaucus	PI 232565	**StH**	3991	4x
kunlunshanicus	CPI 11942	?	1426	6x
scabrus	PI 533217	**StYW**	4967	6x
praecaespitosus	X- 93019	?	6760	6x
lanceolatus	PI 469235	**StH**	2703	4x
dahuricus	T 216	**StHY**	3254	6x
cylindricus	Jinfeng	**StHY**	1984	6x
trachycaulus	PI 636525	**StH**	6764	4x
villosus	PI 531703	?	6765	4x
sibiricus	PI 499464	**StH**	6763	4x
scabrifolius	PI 531544	?	6761	4x
praeruptus	T 217	**StY**	3255	4x
sibiricus	T 215	**StH**	3249	4x
macrochaetus	T 211	**StY**	3249	4x
alatavicus	W 6141	**StYP**	8118	6x
kengii	KJ -328	**StYP**	8128	6x
longiaristatus	PI 401278	**StY**	7302	4x

Table 4. *Hordeum* species having **Xa** and **Xu** genomes.

Genus	Species	Accession #	ID sample	Ploidy
Hordeum	*murinum*	PI 283361	8144	N/A
	murinum	PI 289592	8145	N/A
	murinum	PI 304355	8146	4x
	murinum	PI 304356	8147	N/A
	murinum	PI 304357	8148	4x
	murinum	PI 304358	8149	4x
	murinum	PI 422469	8152	N/A
	murinum	Ciho15683	8139	2x
	murinum ssp glaucum	NGB 6525.2	8173	2x
	murinum ssp murinum	NGB 6526.2	8174	4x
	murinum ssp glaucum	NGB 6528.2	8176	2x
	murinum ssp glaucum	NGB 6535.2	8180	2x
	murinum ssp leporinum	NGB 6529.2	8177	4x
	murinum	PI 206686	8140	4x
	murinum	PI 223373	8141	4x
	murinum	PI 267990	8143	4x
	murinum ssp glaucum	NGB 6846.3	8214	N/A
	murinum ssp glaucum	NGB 6847.3	8215	N/A
	murinum ssp glaucum	NGB 6848.2	8216	2x
	murinum ssp glaucum	NGB 6849.2	8217	2x
	murinum ssp glaucum	NGB 6850.2	8218	2x
	murinum ssp glaucum	NGB 6851.3	8219	2x
	murinum ssp leporinum	NGB 90350.1	8289	4x

murinum ssp leporinum	NGB 90352.1	8290	4x
murinum ssp leporinum	NGB 90353.2	8291	4x
murinum ssp leporinum	NGB 90355.2	8292	4x
murinum ssp murinum	NGB 6530.2	8178	4x
murinum ssp murinum	NGB 6543.2	8188	4x
murinum ssp murinum	NGB 6870.2	8232	4x
murinum ssp leporinum	NGB 90251.2	8281	4x
murinum	Ciho 15683	8139	2x
marinum	PI 247055	8134	2x
marinum	PI 247056	8135	N/A
marinum	PI 283418	8136	N/A
marinum	PI 304353	8137	N/A
marinum	PI 330510	8138	N/A
marinum	PI 401364	8153	2x
marinum	NGB 90606.2	8300	4x
marinum ssp gussoneanum	NGB 6504.2	8154	2x
marinum ssp gussoneanum	NGB 6507.2	8156	4x
marinum ssp gussoneanum	NGB 6508.2	8157	2x
marinum ssp gussoneanum	NGB 6509.2	8158	4x
marinum ssp gussoneanum	NGB 6510.2	8159	4x
marinum ssp gussoneanum	NGB 6512.4	8160	2x
marinum ssp gussoneanum	NGB 6519.2	8167	4x
marinum ssp gussoneanum	NGB 6520.4	8168	4x
marinum ssp gussoneanum	NGB 6521.3	8169	4x
marinum ssp gussoneanum	NGB 6524.2	8172	2x
marinum ssp gussoneanum	NGB 6831.2	8201	2x
marinum ssp gussoneanum	NGB 6832.2	8202	2x
marinum ssp gussoneanum	NGB 6833.2	8203	2x
marinum ssp gussoneanum	NGB 6841.2	8210	4x
marinum ssp gussoneanum	NGB 6843.2	8211	4x
marinum ssp gussoneanum	NGB 6844.1	8212	4x
marinum ssp gussoneanum	NGB 7294.2	8241	2x
marinum ssp gussoneanum	NGB 90031.2	8252	4x
marinum ssp gussoneanum	NGB 90128.2	8256	2x
marinum ssp gussoneanum	NGB 90131.2	8258	4x
marinum ssp gussoneanum	NGB 90237.2	8269	2x
marinum ssp gussoneanum	NGB 90238.2	8270	2x
marinum ssp gussoneanum	NGB 90240.2	8272	2x
marinum ssp gussoneanum	NGB 90241.2	8273	4x
marinum ssp gussoneanum	NGB 90242.2	8274	4x
marinum ssp gussoneanum	NGB 90245.2	8276	2x
marinum ssp gussoneanum	NGB 90249.2	8279	4x
marinum ssp gussoneanum	NGB 90344.2	8283	2x
marinum ssp gussoneanum	NGB 90561.1	8294	2x
marinum ssp gussoneanum	NGB 90562.1	8295	2x

marinum ssp gussoneanum	NGB 90563.1	8296	2x
marinum ssp marinum	NGB 6506.2	8155	2x
marinum ssp marinum	NGB 6513.2	8161	2x
marinum ssp marinum	NGB 6514.2	8162	2x
marinum ssp marinum	NGB 6515.2	8163	2x
marinum ssp marinum	NGB 6516.2	8164	2x
marinum ssp marinum	NGB 6517.2	8165	2x
marinum ssp marinum	NGB 6518.2	8166	2x
marinum ssp marinum	NGB 6820.2	8190	2x
marinum ssp marinum	NGB 6821.2	8191	2x
marinum ssp marinum	NGB 6822.2	8192	2x
marinum ssp marinum	NGB 6823.2	8193	2x
marinum ssp marinum	NGB 6824.2	8194	2x
marinum ssp marinum	NGB 6825.2	8195	2x
marinum ssp marinum	NGB 6826.2	8196	2x
marinum ssp marinum	NGB 6827.2	8197	2x
marinum ssp marinum	NGB 6828.2	8198	2x
marinum ssp marinum	NGB 6829.2	8199	2x
marinum ssp marinum	NGB 6830.2	8200	2x
murinum ssp leporinum	NGB 90350.1	8289	4x
murinum ssp leporinum	NGB 90352.1	8290	4x
murinum ssp leporinum	NGB 90353.2	8291	4x
murinum ssp leporinum	NGB 90355.2	8292	4x
murinum ssp leporinum	NGB 90567.2	8298	4x
murinum ssp leporinum	NGB 90599.2	8299	4x
murinum ssp murinum	NGB 90251.2	8281	4x
murinum ssp murinum	NGB 90565.2	8297	4x
murinum ssp glaucum	NGB 6858.2	8223	2x
murinum ssp glaucum	NGB 6871.2	8233	2x
murinum ssp glaucum	NGB 6872.2	8234	2x
murinum ssp glaucum	NGB 6877.2	8237	2x
murinum ssp glaucum	NGB 7296.2	8243	2x
murinum ssp glaucum	NGB 90133.2	8260	2x
murinum ssp glaucum	NGB 90134.2	8261	2x
murinum ssp glaucum	NGB 90135.2	8262	2x
murinum ssp glaucum	NGB 90252.2	8282	2x
murinum ssp glaucum	NGB 90347.2	8286	2x
murinum sspglaucum	NGB 90348.2	8287	2x
murinum	Ciho 15683	8139	2x
murinum	PI 206686	8140	4x
murinum	PI 223373	8141	N/A
murinum	PI 255142	8142	2x
murinum	PI 267990	8143	4x
marinum ssp marinum	NGB 6845.2	8213	2x
marinum ssp marinum	NGB 7293.2	8240	2x

marinum ssp marinum	NGB 7295.2	8242	2x
marinum ssp marinum	NGB 8551.2	8246	2x
marinum ssp marinum	NGB 8554.2	8247	2x
marinum ssp marinum	NGB 8559.2	8248	4x
marinum ssp marinum	NGB 90126.2	8254	2x
marinum ssp marinum	NGB 90127.2	8255	2x
marinum ssp marinum	NGB 90129.2	8257	2x
marinum ssp marinum	NGB 90153.2	8268	2x
marinum ssp marinum	NGB 90239.2	8271	2x
marinum ssp marinum	NGB 90345.3	8284	2x
marinum ssp marinum	NGB 90346.1	8285	2x

Table 5. *Pseudoroegneria* species.

Species	Accession no.	DNA sample	Ploidy
libanotica	PI 380649	8369	2x
libanotica	PI 222959	8364	2x
libanotica	PI 228391	8365	2x
libanotica	PI 228392	8366	2x
libanotica	PI 229581	8367	2x
spicata	D - 2837	8383	2x
spicata	D - 2838	8384	2x
spicata	D -2844	8385	2x
spicata	PI 739	8387	2x
spicata	PI 232140	8390	2x
spicata	PI 236668	4938	2x
spicata	PI 236681	8391	2x
spicata	KJ 10	8386	2x
spicata	P - 5B	2730	2x
spicata	PI 232127	8388	2x
libanotica	PI 401326	8376	2x
libanotica	PI 380652	8372	2x
libanotica	PI 401321	8374	2x
libanotica	PI 401325	8375	2x
libanotica	PI 401326	8376	2x
ferganensis	PI 3540	7313	2x
cognata	PI 14033	8362	2x
aegilopoides	PI 499637	8355	2x
aegilopoides	PI 565082	8359	2x
aegilopoides	PI 531754	8357	2x
libanotica	PI 401336	8379	2x
libanotica	PI 401339	8381	2x
libanotica	PI 401319	8373	2x
libanotica	PI 380644	8368	2x
libanotica	PI 380650	8370	2x
libanotica	PI 401327	8377	2x

spicata	PI 232134	8389	2x
libanotica	PI 380651	8371	2x
aegilopoides	PI 14037	8360	2x
spicata	MB-36-51-60	2048	2x
spicata	PI 372641	8392	2x
aegilopoides	PI 499638	8356	2x
spicata inermis	Whitmar	8396	2x
libanotica	PI 401331	8378	2x
kuramensis	PI 269864	8363	2x
spicata inermis	PI 236670	5176	2x
ferganensis	T-219	3257	2x

Cyltrimethyl-ammonium bromide (CTAB) DNA extraction

Approximately 100 mg of fresh leaf tissue was collected from each seedling in the greenhouse and placed in 2 ml microcentrifuge tubes containing three steel ball bearings (5mm in diameter). These samples were subsequently frozen in liquid nitrogen and vortexed into fine powder.

One milliliter of extraction buffer, 2% cyltrimethyl-ammonium bromide (CTAB), 1.4 M NaCl, 20 mM ethylenediaminetetra-acetic acid (EDTA), 100 mM Tris-HCl (pH 8.0), 0.2% β-mercapto-ethanol, and 0.1 mg/ml RNase was added to the frozen leaf powder and incubated at 65ºC for 10 min. A 24:1 (v/v) solution of chloroform-isoamyl alcohol was added and mixed vigorously prior to phase separation by centrifugation (14,000 rpm for 10 min) with the Eppendoff centrifuge 5417 R. The upper aqueous phase containing nucleic acid was transferred to a 1.5 ml microcentrifuge tube and mixed with 0.7 ml of cold isopropanol. Nucleic acids were hooked out with a glass pipette, transferred to a new tube, and washed twice in a solution of 70% ethanol. Samples were air dried and vacuumed dry for 5 min and then dissolved in TE Buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0) (see Appendix). Genomic DNA was quantified by Nanodrop

Spectrophotometer ND-1000 (Nanodrop Technologies, Rockland, DE) at the wavelength of 260 nm. The genomic DNA was adjusted and normalized to 40 ng/µl with sterilized double distilled water. DNA was evaluated by using 2% agarose gel stained with ethidium bromide (5 mg/ml) and visualized under UV light, then photographed in imaging system UVP 2UV Transilluminator (UVP, Inc, Upland, CA).

Qiagen DNeasy 96-well plate protocol

This second method of isolation of genomic DNA from fresh leaf tissue was used only in *Hordeum* species. Fresh, youngest leaves from various plants were harvested and weighted. Fifty milligrams of each sample was put into collection racks on ice. One tungsten carbide bead was added to each well. Buffer AP1 and Reagent DX were preheated to 65ºC in a water bath. Four hundred milliliters of the master mix containing 45 ml of API Buffer, 112.5 µl of RNase A (100 mg/ml), and 112.5 µl of Reagent DX were delivered to each well containing sample. The samples were disrupted in the Mixer Mill set a 30 Hz for $1\frac{1}{2}$ min, then, removed from the Mixer plates and reassembled in reverse order then disrupted for an additional $1\frac{1}{2}$ min. This step was the most critical because the longer time could shear DNA. The plate collection racks were placed in the centrifuge and spun up to 3000 rpm. One hundred and thirty milliliters of AP2 Buffer was added to each sample then recapped with new caps. The collection racks were shaken vigorously by hand, up and down, for 15 sec. They were centrifuged and spun up to 3000 rpm for 5 min. The racks were rotated in the centrifuge and spun for an additional 3 min at 6000 rpm. Carefully, 400 µl of the supernatant was transferred into newly labeled racks of collection tubes. A volume of 600 µl of AP3/E Buffer was added

and recapped with new caps. The collection racks were shaken vigorously by hand, up and down, for 15 sec. Collection racks were centrifuged and spun up to 3000 rpm. Labeled DNeasy plate was placed on top of the S-Block. One milliliter of the solution was transferred to DNeasy plate and sealed with Airpore sheets, and then centrifuged for 4 min at 6000 rpm. The airpore sheets were removed and the 400 µl of AW Buffer was added to each sample. To dry the DNeasy membranes, the samples were centrifuged for 15 min at 6000 rpm. Airpore tape was used to prevent cross contamination between the samples during the centrifugation and the caps were removed and discarded after each centrifugation step. To release the DNA 50 µl of elution AE buffer was added at room temperature into DNeasy plates, sealed, and were incubated for 1 min, then centrifuged for 2 min at 6000 rpm. The plate was labeled DNA collected and used for PCR analysis.

The PCR reaction mixture was composed of 10 µl Buffer 10X, 0.2 mM of each deoxynucleotide 25 mM $MgCl_2$, 10 mM dNTP, 1U Taq polymerase, 4 µl of each primer (Table 6; forward and reverse), and 200 ng Template DNA in a final volume of 25 µl. Another reagent, GoTaq Green master mix 2X, was compared with the first. This master mix including $MgCl_2$, dNTP and Taq polymerase was added to the DNA Template and then adjusted with nuclease-free water to the desired final volume of 25µl. PCR was performed in the Applied Bio System 9700 Thermocycler.

The initial denaturation occurred at 95˚C for $2\frac{1}{2}$ min. The complete cycle consisted of a denaturing step of 95ºC for $2\frac{1}{2}$ min, an annealing step of 55ºC for 30 sec and an extension step of 72ºC for 5 min. The reaction mixture was allowed to run for 30 cycles.

Table 6. Primer sequences used (www.operon.com).

Primer names	Primer sequences 5' to 3'	Tm (ºC)	Size (bp)
D15F	GTGCGGTGCGTCATAGA	62.32	498
D15R	ATCCGTGCTTAGAAAGGTAGCA	60.81	498
B04F	GGACTGGAGTTCAGAGCAATC	62.57	341
BO4R	GGACTGGAGTAGCTTTTCAAACA	60.99	341
B14F1	TCCGCTCTCGGGATGTGAC	62.18	269
B14R1	TCCTGAAGGTAAAACTTTCTGTTTTTT	58.28	269
NO5F	GCCATCGAGACCTATGCAAT	60.4	520
NO5R	ACTGAACGCCAAAGTGCG	59.9	520

Gel electrophoresis

Electrophoresis gel was used to confirm the size and the presence of the PCR product. The PCR product was mixed with 10X loading dye solution, 7 µl of the total 25 µl PCR reaction, and 3 µl of loading dye, and then analyzed by electrophoresis on a 2% agarose gel to confirm the presence of DNA. The DNA was photographed in imaging system UVP, 2UV Transilluminator. The size of the fragments was estimated using 100 bp ladders (see Appendix). All reactions were performed in triplicate and only the positive bands were considered for this study.

Chromosome count analysis

The roots were pretreated in 5 ml of a saturated solution of P-dichlorobenzene at 4ºC for 4 hours and fixed in 5 ml of Carnoy's fixative (ethanol-chloroform-acetic acid). After being softened with 1.5% pectinase (Sigma) for 1 hour at room temperature and

hydrolysed in 1 N HCl at 60ºC for 10 min, chromosomes were stained in basic fucshin and squashed in 2% (W/V) acetic orcein.

DNA cloning and sequencing

Plant materials selected for cloning and sequencing are listed in Table 7.

Cloning and sequencing of the STS Y marker

Primers used for the STS **Y** marker (Table 8) were based on the RAPD marker JC51 identified in the laboratory of Dr. Richard Wang at the United States Department of Agriculture (USDA), Agriculture Research Service (ARS) in Logan, Utah.

The genomic DNA isolated from plants was used for PCR amplification of the STS marker. Leaf tissue samples were collected from several individual plants for each accession and were frozen in liquid nitrogen. DNA was extracted following the CTAB and Qiagen DNeasy 96-well plate methods. PCR mixtures of total volume of 25 µl, containing 2 ng template DNA, and 12.5 µl of Go Taq Green master mix 2X, 4 µl of primer BO4 forward and reverse primers (Operon). Amplification was performed in DNA Thermocycler. The PCR program consisted of pre-denaturation at 95ºC for 2½ min, 30 cycles of 95ºC for 1 min, annealing at 55ºC for 30 sec and extension at 72ºC for 1 min followed by a final extension for 5 min. The PCR fragments were electrophoresed in 2% agarose gels stained with 0.5 mg/ml ethidium bromide and visualized under UV light. PCR reactions producing appropriate size of DNA band were purified for cloning.

Table 7. Plant materials selected for cloning and sequencing.

Name of species	ID #	Accession #	Ploidy	Origin
P. ferganensis	T219	3257	2x	Tajikistan, Russian
P. spicata	PI 232134	8389	2x	Wyoming, USA.
P. libanotica	PI 401326	8376	2x	Iran
H. marinum	NGB 90249.2	8279	4x	Greece via Sweden
H. murinum ssp lepor	NGB 90251.2	8281	4x	Greece via Sweden
H. murinum	PI 206686	8140	4x	Turkey via Aberdeen
H. murinum ssp lepor	NGB 90350.1	8289	4x	Spain via Sweden
E. longiaristatus	PI 401282	5992	4x	KBJ

Table 8. Primers used in searching for the Y-genome (www.operon.com).

Primer names	Nucleotide sequences
BO4 forward	5'-TCCGCTCTGGGATGTGAC-3'
BO4 reverse	5'-AAAACAGAAAGTTTTACCTTCAGGA -3'

PCR product purification

This procedure was done by adding 5:1 ratio, 5 volumes of PB1 Buffer to 1 volume of the PCR product. To bind the DNA, the QIAquick spin columns were placed into collection tubes and centrifuged for 60 sec. The flow trough was discarded and QIAquick columns were placed back and centrifuged for additional 60 sec. PE buffer (0.75 ml) was added to wash the DNA and centrifuged for 60 sec. The DNA was eluted by adding 35 µl of Buffer EB (10 mM Tris-Cl, pH 8.5) to the center of the QIAquick membrane and centrifuged for 60 sec. The purified DNA was analyzed on 1% of agarose

gel and visualized under UV light. The DNA was photographed in imaging system UVP, 2UV Transilluminator.

Cloning reactions

Purified fragments were ligated and cloned into pSC-A vector (Stratagene, La Jolla, CA) and transformed into *Escherichia coli* (StraClone Solopack competent cells).

After ligation, 1 µl of the reaction was added to thawed StrataClone Solopack competent cells and mixed and incubated for 20 min on ice. The reaction was heated shock at 42ºC for 45 sec and incubated for 2 min on ice. To allow the competent cells to recover, 250 µl of SOC (see Appendix) medium was pre-warmed, and added to the reaction and agitated at 37ºC for 1 hour. LB-ampicilin plates were prepared by spreading 40 µl of 2% X-gal on each plate. Twenty-five to 350 µl of the transformation mixture was plated on the LB ampicillin-X gal plates (see Appendix) and incubated overnight at 37ºC. The formation of blue or white colonies was observed the following day.

The white or light blue colonies were selected for PCR reaction using M13 (forward and reverse) primers (Table 9), and the colonies that contained the correct sizes of DNA were cultured in the shaker overnight at 37ºC.

Table 9. Primers used to amplify cloned insert DNA (www.operon.com).

Primers	Primer sequence (5' to 3')
M13 Forward	GGAAACAGCTATGACCATG
M13 Reverse	ACTGGCCGTCGTTTTACAA

Plasmid DNA Extraction

Plasmid DNA preparation was performed using QIAprep Miniprep (Qiagen, Valencia, CA). The cells were collected and suspended in 250 µl Buffer P1 and transferred to new microcentrifuge tubes. To mix DNA, 250 µl of Buffer P2 was added and gently inverted 4-6 times until the solution became viscous and slightly clear. Then, 350 µl of N3 Buffer was added and centrifuged for 10 min at 13000 rpm. The supernatant was collected and transferred to QIAprep spin column, then centrifuged for 60 sec. To wash the DNA, PE Buffer (0.75 ml) was added and the column was centrifuged for 60 sec; the flow-through was discarded and the column was centrifuged for an additional 60 sec. To elute the DNA, 35 µl of EB Buffer (10 mM Tris-Cl, pH 8.5) was added in the center of each QIAprep pin column and centrifuged for 60 sec. The plasmid DNA was quantified by Nanodrop Spectrophotometer ND-1000 at the wavelength of 260 nm. The plasmid DNA concentration was adjusted to 50-100 ng/µl with sterilized double distilled water. Plasmid DNA was sequenced on an Applied BioSystems 3730 (DNA Analyzer) automated sequencer (Applied BioSystems, Foster City, CA) at the Center of Integrated BioSystems, Utah State University.

RESULTS

Polymerase Chain Reaction for STS marker of St and Y-genomes in *Elymus* species

The objective of this work was to find the diploid donor of the **Y** genome. About two hundred twelve accessions of *Elymus, Hordeum*, and *Pseudoroegneria* were used in this study. The accessions were tested for the presence or absence of the **St** and **Y** genomes. A Polymerase Chain Reaction (PCR) method was used to amplify genome-specific Sequence Tagged Site (STS) markers from the genomic DNA. *Agropyron* and *Australopyrum* species were used as negative controls, because they possess the **P** and **W** genomes, respectively. *Elymus longiaristatus* PI 401282 (**StY**) was used as the positive control. Most of the species in the *Elymus* genus are tetraploid (2n = 4x = 28) and hexaploids (2n = 6x = 42). The test for the **St** genome was carried out using three STS markers:

1) B04F/R-fragment size 341 base pairs;
2) D15F/R- fragment size 498 base pairs;
3) N05F/R- fragment size 520 base pairs.

The primers pair B14F1/R1, producing a fragment of 269 base pairs, was used for testing the presence of the **Y** genome.

Results of these PCR reactions are presented in Table 10. As expected, all three STS-PCR tests were negative for *Hordeum bogdanii* (**H** genome), *Agropyron cristatum* (**P**), and *Australopyrum retrofractum* (**W**). All *Elymus* species are supposed to have the **St** genome. However, some STS-PCR tests failed to show the presence of the **St** genome in some *Elymus* species. *Elymus fibrosus, E. tsukushiensis,* and *E. praeruptus* had

negative reactions from all three STS-PCR tests for the **St** genome. All other *Elymus* species tested in this study had at least one positive STS-PCR even though some species yielded variant PCR products, i.e. the fragments were of unexpected lengths. Only *E. tianshanicus* and *E. alatavicus* were positive for all three **St** genome tests and yielded STS markers of the expected sizes.

The primer pair B14F1/R1 yielded one fragment of the expected size, 269 bp, in 22 out of 23 *Elymus* accessions that are known to have the **Y** genome. Only the **StY** *E. praeruptus* failed to produce positive result. The following *Elymus* species were negative in all three replicated PCR tests for the **Y** genome: *E. arizonicus*, *E. canadenisis*, *E. caninus, E. lanceolatus*, *E. trachycaulus*, *E. sibiricus***,** *E. scabrifolius*, and *E. glaucus***.** These species contain **St** and **H** genomes (Table 10), thus the negative result was expected. Only one **StH** tetraploid *Elymus* species, *E. fibrosus*, yielded the unexpected positive result with the **Y** genome test.

Ten *Elymus* species in this study had unknown genome constitutions. Five of these species were positive for the **Y** genome test: *E. curvatus*, *E. tianshanicus*, *E. laxiflorus*, *E. kunlunshanicus,* and *E. praecaespitosus*. The other five *Elymus* species, *E. angustiglumis*, *E. interruptus, E. scabriglumis, E. villosus*, and *E. scabrifolius*, were negative with the STS-PCR for the **Y** genome.

Polymerase Chain Reaction for STS marker of Y genome in *Hordeum* species

A total of 126 accessions of *Hordeum marinum* and *H. murinum* were analyzed for the **Y** genome STS marker. Twelve of these were tested positive for the **Y** genome

Table 10. Results of STS-PCR test of Triticeae species using primer pairs designed for genome-specific STS marker of the **St** and **Y** genomes. Each PCR test was run in three replications. Numbers in parentheses represent fragments of unexpected sizes: 1 = 600bp, 2 = 700bp, 3 = 800bp and 4 = 1000bp.)

					St genome			**Y** genome
Genus	Species	Accession#	Genome symbols	Ploidy	B04F B04R 341bp	D15F D15R 498bp	N05F N05R 520bp	B14F1 B14R1 269bp
Pseudoroegneria	*spicata*	PI 610987	**St**	2x	-	+(1,4)	+	-
Pseudoroegneria	*stipifolia*	PI 313960	**St**	2X	-	+(1)	+	-
Hordeum	*bogdanii*	PI 269406	**H**	2x	-	-	-	-
Agropyron	*cristatum*	PI 499389	**P**	2x	-	-	-	-
Australopyron	*retrofractum*	PI 531553	**W**	2x	-	-	-	-
Elymus	*longiaristatus*	PI 401282	**StY**	4x	+	+	-	+
	arizonicus	PI 531558	**StH**	4x	-	+(1)	-	-
	canadenisis	PI 531565	**StH**	4x	-	+(1)	+	-
	caucasicus	PI 531572	**StY**	4x	+	-	+(4)	+
	batalinii	PI 314462	**StPY**	6x	+	+	+(4)	+
	kengii	PI 504457	**StPY**	6x	+	+(2)	+(4)	+
	villifer	KJ-174	**StHY**	6x	+	+(4)	-	+
	excelsus	W 94039	**StHY**	6x	+	+	-	+
	rectisetus	PI 533028	**StYW**	6x	+	+	+(4)	+
	drobovii	PI 314196	**StHY**	6x	+	+	-	+
	curvatus	PI 531579	?	4x	+	+(2)	+(4)	+
	angustiglumis	PI 531639	?	4x	+	+(1,3)	+	-
	tianshanicus	T 210	?	6x	+	+	+	+
	tschimganicus	PI 564498	**StStY**	6x	+	+	+(4)	+
	fibrosus	PI 547320	**StH**	4x	-	-	-	+
	gmelinii	AJC 266	**StY**	4x	+	+	-	+
	elymoides	TAJ 90401	**StH**	4x	-	+(1)	-	-
	nevski	H-10215	**StY**	4x	+	+	-	+
	alatavicus	PI 531709	**StPY**	6x	+	+	-	+
	caninus	PI 547706	**StH**	4x	+	+(1)	-	-
	interruptus	PI 531617	?	4x	+	+(1)	-	-
	laxiflorus	KJ 278	?	6x	+	+(1)	-	+
	scabriglumis	PI 202147	?	6x	-	+	-	-
	tangutorus	CPI 11975	**StHY**	6x	+	+	-	+
	tsukushiensis	PI 499624	**StHY**	6x	-	-	-	+
	nutans	PI 531587	**StHY**	6x	+	+(2)	+(2)	+
	glaucus	PI 232565	**StH**	4x	-	+(1)	+	-
	kunlunshanicus	CPI 11942	?	6x	+	+	-	+
	scabrus	PI 533217	**StYW**	6x	+	+	+(4)	+
	praecaespitosus	X- 93019	?	6x	-	+	+	+
	lanceolatus	PI 469235	**StH**	4x	-	+(1)	+	-
	dahuricus	T 216	**StHY**	6x	+	+	-	+
	cylindricus	Jinfeng	**StHY**	6x	+	+	-	+
	trachycaulus	PI 636525	**StH**	4x	-	-	+	-
	villosus	PI 531703	?	4x	-	+	+	-

sibiricus	PI 499464	**StH**	4x	-	+(1)	+	-
scabrifolius	PI 531544	?	4x	-	+(1)	+	-
praeruptus	T 217	**StY**	4x	-	-	-	-
sibiricus	T 215	**StH**	4x	+	+(1)	+	-
macrochaetus	T 211	**StY**	4x	+	+(1)	+	+
alatavicus	W 6141	**StYP**	6x	+	+	+	+
kengii	KJ -328	**StYP**	6x	+	+(1)	+(4)	+
longiaristatus	PI 401278	**StY**	4x	+	+	+(4)	+

Table 11. Results of STS-PCR test of *Hordeum* species using primer pair designed for genome-specific STS marker of the **Y** genome.

					Y genome
Genus	Species	Accession #	DNA sample	Ploidy	B14F1 B14R1 269bp
Elymus	*longiaristatus*	PI 401282	StY5992	4x	++
Hordeum	*murinum*	PI 283361	8144	na	-
	murinum	PI 289592	8145	na	-
	murinum	PI 304355	8146	4x	-
	murinum	PI 304356	8147	na	-
	murinum	PI 304357	8148	4x	-
	murinum	PI 304358	8149	4x	-
	murinum	PI 422469	8152	na	+
	murinum	Ciho 15683	8139	2x	-
	murinum ssp glaucum	NGB 6525.2	8173	2x	-
	murinum ssp murinum	NGB 6526.2	8174	4x	-
	murinum ssp glaucum	NGB 6528.2	8176	2x	-
	murinum ssp glaucum	NGB 6535.2	8180	2x	-
	murinum spp leporinum	NGB 6529.2	8177	4x	-
	murinum	PI 206686	8140	4x	+
	murinum	PI 223373	8141	4x	-
	murinum	PI 267990	8143	4x	-
	murinum ssp glaucum	NGB 6846.3	8214	na	-
	murinum ssp glaucum	NGB 6847.3	8215	na	-
	murinum ssp glaucum	NGB 6848.2	8216	2x	-
	murinum ssp glaucum	NGB 6849.2	8217	2x	-
	murinum ssp glaucum	NGB 6850.2	8218	2x	-
	murinum ssp glaucum	NGB 6851.3	8219	2x	-
	murinum ssp leporinum	NGB 90350.1	8289	4x	-
	murinum ssp leporinum	NGB 90352.1	8290	4x	-
	murinum ssp leporinum	NGB 90353.2	8291	4x	+
	murinum ssp leporinum	NGB 90355.2	8292	4x	-
	murinum ssp murinum	NGB 6530.2	8178	4x	+
	murinum ssp murinum	NGB 6543.2	8188	4x	-
	murinum ssp murinum	NGB 6870.2	8232	4x	+
	murinum ssp leporinum	NGB 90251.2	8281	4x	+

murinum	Ciho 15683	8139	2x	-
marinum	PI 247055	8134	2x	-
marinum	PI 247056	8135		-
marinum	PI 283418	8136	na	-
marinum	PI 304353	8137	na	-
marinum	PI 330510	8138	na	-
marinum	PI 401364	8153	2x	-
marinum	NGB 90606.2	8300	4x	+
marinum sspgussoneanum	NGB 6504.2	8154	2x	-
marinum ssp gussoneanum	NGB 6507.2	8156	4x	-
marinum ssp gussoneanum	NGB 6508.2	8157	2x	-
marinum ssp gussoneanum	NGB 6509.2	8158	4x	-
marinum ssp gussoneanum	NGB 6510.2	8159	4x	-
marinum ssp gussoneanum	NGB 6512.4	8160	2x	-
marinum ssp gussoneanum	NGB 6519.2	8167	4x	-
marinum ssp gussoneanum	NGB 6520.4	8168	4x	-
marinum ssp gussoneanum	NGB 6521.3	8169	4x	-
marinum ssp gussoneanum	NGB 6524.2	8172	2x	-
marinum ssp gussoneanum	NGB 6831.2	8201	2x	-
marinum ssp gussoneanum	NGB 6832.2	8202	2x	-
marinum ssp gussoneanum	NGB 6833.2	8203	2x	-
marinum ssp gussoneanum	NGB 6841.2	8210	4x	-
marinum ssp gussoneanum	NGB 6843.2	8211	4x	-
marinum ssp gussoneanum	NGB 6844.1	8212	4x	-
marinum ssp gussoneanum	NGB 7294.2	8241	2x	-
marinum ssp gussoneanum	NGB 90031.2	8252	4x	-
marinum ssp gussoneanum	NGB 90128.2	8256	2x	-
marinum ssp gussoneanum	NGB 90131.2	8258	4x	-
marinum ssp gussoneanum	NGB 90237.2	8269	2x	-
marinum ssp gussoneanum	NGB 90238.2	8270	2x	-
marinum ssp gussoneanum	NGB 90240.2	8272	2x	-
marinum ssp gussoneanum	NGB 90241.2	8273	4x	-
marinum ssp gussoneanum	NGB 90242.2	8274	4x	-
marinum ssp gussoneanum	NGB 90245.2	8276	2x	-
marinum ssp gussoneanum	NGB 90249.2	8279	4x	+
marinum ssp gussoneanum	NGB 90344.2	8283	2x	-
marinum ssp gussoneanum	NGB 90561.1	8294	2x	-
marinum ssp gussoneanum	NGB 90562.1	8295	2x	-
marinum ssp gussoneanum	NGB 90563.1	8296	2x	-
marinum ssp marinum	NGB 6506.2	8155	2x	
marinum ssp marinum	NGB 6513.2	8161	2x	-
marinum ssp marinum	NGB 6514.2	8162	2x	-
marinum ssp marinum	NGB 6515.2	8163	2x	-
marinum ssp marinum	NGB 6516.2	8164	2x	-
marinum ssp marinum	NGB 6517.2	8165	2x	-
marinum ssp marinum	NGB 6518.2	8166	2x	-
marinum ssp marinum	NGB 6820.2	8190	2x	-
marinum ssp marinum	NGB 6821.2	8191	2x	-
marinum ssp marinum	NGB 6822.2	8192	2x	-

marinum ssp marinum	NGB 6823.2	8193	2x	-
marinum ssp marinum	NGB 6824.2	8194	2x	-
marinum ssp marinum	NGB 6825.2	8195	2x	-
marinum ssp marinum	NGB 6826.2	8196	2x	-
marinum ssp marinum	NGB 6827.2	8197	2x	-
marinum ssp marinum	NGB 6828.2	8198	2x	-
marinum ssp marinum	NGB 6829.2	8199	2x	-
marinum ssp marinum	NGB 6830.2	8200	2x	-
murinum ssp leporinum	NGB 90350.1	8289	4x	+
murinum ssp leporinum	NGB 90352.1	8290	4x	+
murinum ssp leporinum	NGB 90353.2	8291	4x	+
murinum ssp leporinum	NGB 90355.2	8292	4x	-
murinum ssp leporinum	NGB 90567.2	8298	4x	-
murinum ssp leporinum	NGB 90599.2	8299	4x	+
murinum ssp murinum	NGB 90565.2	8297	4x	-
murinum ssp glaucum	NGB 6858.2	8223	2x	-
murinum ssp glaucum	NGB 6871.2	8233	2x	-
murinum ssp glaucum	NGB 6872.2	8234	2x	-
murinum ssp glaucum	NGB 6877.2	8237	2x	-
murinum ssp glaucum	NGB 7296.2	8243	2x	-
murinum ssp glaucum	NGB 90133.2	8260	2x	-
murinum ssp glaucum	NGB 90134.2	8261	2x	-
murinum ssp glaucum	NGB 90135.2	8262	2x	-
murinum ssp glaucum	NGB 90252.2	8282	2x	-
murinum ssp glaucum	NGB 90347.2	8286	2x	-
murinum ssp glaucum	NGB 90348.2	8287	2x	-
murinum	Ciho 15683	8139	2x	-
murinum	PI 255142	8142	2x	-
murinum	PI 267990	8143	4x	-
marinum ssp marinum	NGB 6845.2	8213	2x	-
marinum ssp marinum	NGB 7293.2	8240	2x	-
marinum ssp marinum	NGB 7295.2	8242	2x	-
marinum ssp marinum	NGB 8551.2	8246	2x	-
marinum ssp marinum	NGB 8554.2	8247	2x	-
marinum ssp marinum	NGB 8559.2	8248	4x	-
marinum ssp marinum	NGB 90126.2	8254	2x	-
marinum ssp marinum	NGB 90127.2	8255	2x	-
marinum ssp marinum	NGB 90129.2	8257	2x	-
marinum ssp marinum	NGB 90153.2	8268	2x	-
marinum ssp marinum	NGB 90239.2	8271	2x	-
marinum ssp marinum	NGB 90345.3	8284	2x	-
marinum ssp marinum	NGB 90346.1	8285	2x	-

Xa genome in *H. marinum*
Xu genome in *H. murinum*

marker, but all of them are tetraploids (Table 11). Thus, all the diploid accessions of the two *Hordeum* species were negative for the **Y** genome.

Polymerase Chain Reaction for STS marker of St and Y genomes in *Pseudoroegneria* species

Forty-one accessions of *Pseudoroegneria* were tested for the **Y-** and **St-**genome STS markers (Table 12). The results show that all the *Pseudoroegneria* species on the list (Table 12) possess the **St** genome, with the two exceptions of *P. libanotica* (PI 401325 and PI 401331). Three out of forty-one accessions of *Pseudoroegneria* species contained the **Y** genome. All these three accessions (*E. libanotica* PI 401326, *E. spicata* PI 232134, and *E. ferganensis* T-219) were positive with all three STS-PCR tests for the **St** genome.

Intensities of amplified Y genome STS marker in selected accessions

To verify the results of **Y** genome marker amplification, template DNA concentration was normalized according to ploidy levels of tested species. Results are shown in Fig. 7. The three diploid *Pseudoroegneria* species and two tetraploid *Hordeum* species previously tested positive were included in this test along with species known to have or lack the **Y** genome. The intensity of the marker band was much stronger in species known to have the **Y** genome than that in species having other genomes.

Chromosome counts

To confirm the ploidy level of the *Pseudoroegneria* species that are putative donor of the **Y** genome, chromosome count was carried out for *P. ferganensis* T-219 (**St**),

Table 12. Results of STS-PCR test of *Pseudoroegneria* species using primer pairs designed for genome-specific STS markers of the **St** and **Y** genomes.

Species	Accession #	DNA sample	Ploidy	**Y** genome	**St** genome		
				B14R1 B14F1 269bp	B04F B04R 341bp	D15F D15R 498bp	N05F N05R 520bp
libanotica	PI 380649	8369	2x	-	-	+	-
libanotica	PI 222959	8364	2x	-	-	+	-
libanotica	PI 228391	8365	2x	-	-	+	-
libanotica	PI 228392	8366	2x	-	+	+	-
libanotica	PI 229581	8367	2x	-	+	+	-
spicata	D - 2837	8383	2x	-	+	+	+
spicata	D - 2838	8384	2x	-	+	+	+
spicata	D -2844	8385	2x	-	+	+	+
spicata	PI 739	8387	2x	-	+	+	+
spicata	PI 232140	8390	2x	-	+	+	+
spicata	PI 236668	4938	2x	-	+	+	+
spicata	PI 236681	8391	2x	-	+	+	+
spicata	KJ 10	8386	2x	-	-	+	+
spicata	P - 5B	2730	2x	-	+	+	+
spicata	PI 232127	8388	2x	-	-	+	+
libanotica	PI 401326	8376	2x	+	+	+	+
libanotica	PI 380652	8372	2x	-	-	+	-
libanotica	PI 401321	8374	2x	-	-	+	-
libanotica	PI 401325	8375	2x	-	-	-	-
ferganensis	PI 3540	7313	2x	-	+	+	-
cognata	PI 14033	8362	2x	-	+	+	+
aegilopoides	PI 499637	8355	2x	-	+	+	+
aegilopoides	PI 565082	8359	2x	-	+	+	+
aegilopoides	PI 531754	8357	2x	-	+	+	+
libanotica	PI 401336	8379	2x	-	+	+	+
libanotica	PI 401339	8381	2x	-	+	+	-
libanotica	PI 401319	8373	2x	-	+	+	-
libanotica	PI 380644	8368	2x	-	+	+	-
libanotica	PI 380650	8370	2x	-	+	+	+
libanotica	PI 401327	8377	2x	-	+	+	-
spicata	PI 232134	8389	2x	+	+	+	+
libanotica	PI 380651	8371	2x	-	+	+	-
aegilopoides	PI 14037	8360	2x	-	-	+	-
spicata	MB-36-51-60	2048	2x	-	-	+	+
spicata	PI 372641	8392	2x	-	-	+	+
aegilopoides	PI 499638	8356	2x	-	-	+	+
spicata inermis	Whitmar	8396	2x	-	-	+	+
libanotica	PI 401331	8378	2x	-	-	-	-
kuramensis	PI 269864	8363	2x	-	-	+	+
spicata inermis	PI 236670	5176	2x	-	-	+	+
ferganensis	T-219	3257	2x	+	+	+	+

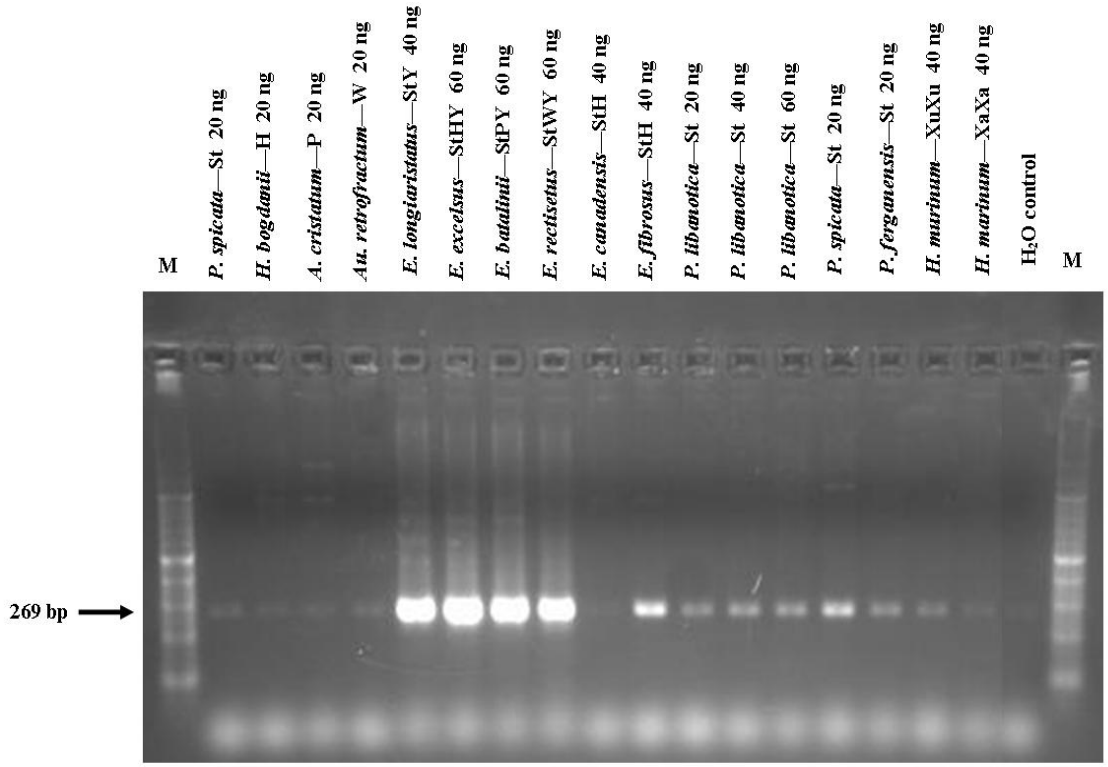

Fig. 7. Amplified **Y** genome STS marker. B14 F1/R1-269bp, in species of *Pseudoroegneria, Hordeum, Agropyron, Australopyrum,* and *Elymus*. Template DNA was normalized according to ploidy levels so that each genome was approximately 20 ng. Three DNA concentrations of diploid *P. libanotica* were used to show that 30 cycles of PCR amplification was appropriate for the test**.** Note the similar band intensity between *E. fibrosus* (lane 11) and second accession of *P. spicata* (lane 15). Lanes M (1 & 20) contains the 100-bp DNA ladders as size markers.

P. spicata PI 232134 (St) and *P. libanotica* PI 401326 (St). The chromosome count results showed that these accessions are diploid (2n = 2x = 14).

Cloning and sequencing DNA from *Pseudoroegneria* and *Hordeum*

The amplified B14F1/R1- 269-bp products (Table 13) from the genomic DNA of suspected **Y**-genome donor species were cloned and sequenced. These sequences were compared and aligned to determine their similarities to the **Y** genome's random amplified

polymorphic DNA (RAPD) marker, JC51. After trimming off the primers, DNA sequences of cloned PCR products were analyzed with programs available at the European Molecular Biology Laboratory (EMBL) website and DNAstar (Lasergene® 7 Software, Madison WI) at USU Stress Physiology Laboratory. Phylogenic analyses were performed by grouping all nucleotides. Multiple alignments were performed using the CLUSTAL W program (EMBL).

Table 13. Sequence alignments. CLUSTAL W (1.83) Multiple Sequence Alignments (Sequence type explicitly set to DNA, Sequence format is Pearson).

Sequence Number	Name	Size
Sequence 1	JC51 Y marker	271 bp
Sequence 2	8389-01-St-spicata	270 bp
Sequence 3	8389-03-St-spicata	270 bp
Sequence 4	8389-04-St-spicata	270 bp
Sequence 5	8389-05-St-spicata	270 bp
Sequence 6	8389-07-St-spicata	269 bp
Sequence 7	8389-08-St-spicata	269 bp
Sequence 8	8389-10-St-spicata	269 bp
Sequence 9	8389-16-St-spicata	270 bp
Sequence 10	8389-19-St-spicata	269 bp
Sequence 11	8389-21-St-spicata	270 bp
Sequence 12	8389-23-St-spicata	269 bp
Sequence 13	8389-24-St-spicata	270 bp
Sequence 14	3257-03-St-ferganensis	270 bp
Sequence 15	3257-02-St-ferganensis	270 bp
Sequence 16	3257-06-St-ferganensis	270 bp
Sequence 17	3257-19-St-ferganensis	270 bp
Sequence 18	3257-23-St-ferganensis	269 bp
Sequence 19	8281-01-Xu-murinum	270 bp
Sequence 20	8281-02-Xu-murinum	270 bp
Sequence 21	8281-03-Xu-murinum	270 bp

Sequence 22	8281-04-Xu-murinum	270 bp
Sequence 23	8281-06-Xu-murinum	270 bp
Sequence 24	8281-07-Xu-murinum	271 bp
Sequence 25	8281-09-Xu-murinum	270 bp
Sequence 26	8281-12-Xu-murinum	270 bp
Sequence 27	8281-13-Xu-murinum	270 bp
Sequence 28	8281-15-Xu-murinum	268 bp
Sequence 29	8281-16-Xu-murinum	270 bp
Sequence 30	8281-17-Xu-murinum	270 bp
Sequence 31	8281-20-Xu-murinum	270 bp
Sequence 32	8281-22-Xu-murinum	270 bp
Sequence 33	8281-23-Xu-murinum	270 bp
Sequence 34	8281-25-Xu-murinum	265 bp
Sequence 35	8281-27-Xu-murinum	270 bp
Sequence 36	8281-28-Xu-murinum	268 bp
Sequence 37	8140-01-Xu-murinum	270 bp
Sequence 38	8140-03-Xu-murinum	270 bp
Sequence 39	8140-04-Xu-murinum	270 bp
Sequence 40	8140-07-Xu-murinum	270 bp
Sequence 41	8140-09-Xu-murinum	268 bp
Sequence 42	8140-10-Xu-murinum	270 bp
Sequence 43	8140-11-Xu-murinum	270 bp
Sequence 44	8140-34-Xu-murinum	269 bp
Sequence 45	8140-35-Xu-murinum	270 bp
Sequence 46	8299-01-Xu-murinum	270 bp
Sequence 47	8299-06-Xu-murinum	270 bp
Sequence 48	8299-08-Xu-murinum	270 bp
Sequence 49	8299-09-Xu-murinum	267 bp
Sequence 50	8299-11-Xu-murinum	270 bp
Sequence 51	8299-17-Xu-murinum	270 bp
Sequence 52	8299-18-Xu-murinum	270 bp
Sequence 53	8299-19-Xu-murinum	270 bp
Sequence 54	8299-20-Xu-murinum	270 bp
Sequence 55	8299-21-Xu-murinum	270 bp

Sequence 56	8299-22-Xu-murinum	270 bp
Sequence 57	8299-24-Xu-murinum	270 bp
Sequence 58	5992-5-StY-E.longi	269 bp
Sequence 59	5992-9-StY-E.longi	270 bp
Sequence 60	5992-10-StY-E.longi	269 bp
Sequence 61	5992-14-StY-E.longi	270 bp
Sequence 62	5992-16-StY-E.longi	240 bp
Sequence 63	5992-17-StY-E.longi	269 bp
Sequence 64	5992-18-StY-E.longi	270 bp
Sequence 65	5992-20-StY-E.longi	269 bp
Sequence 66	8376-01-St-libanotica	270 bp
Sequence 67	8376-02-St-libanotica	270 bp
Sequence 68	8376-03-St-libanotica	269 bp
Sequence 69	8376-04-St-libanotica	270 bp
Sequence 70	8376-06-St-libanotica	269 bp
Sequence 71	8376-08-St-libanotica	269 bp
Sequence 72	8376-09-St-libanotica	270 bp
Sequence 73	8279-04-Xa-marinum	270 bp
Sequence 74	8279-05-Xa-marinum	267 bp
Sequence 75	8279-06-Xa-marinum	270 bp
Sequence 76	8279-08-Xa-marinum	270 bp
Sequence 77	8279-09-Xa-marinum	270 bp
Sequence 78	8279-10-Xa-marinum	270 bp
Sequence 79	8279-11-Xa-marinum	270 bp

Seventy-nine sequences were analyzed including the **Y**-genome RAPD marker, JC51 (GenBank accession BV679236), which was amplified from *E. rectisetus* (**StWY**). Twelve clones of plasmid DNA sequence were isolated from *P. spicata* (**St**), five from *P. ferganensis* (**St**), seven from *P. libanotica* (**St**), thirty nine from *Hordeum murinum* (**Xu**), seven from *H. marinum* (**Xa**), and eight from the positive control, *Elymus longiaristatus* (**StY**). The phylogenic tree was based on the CLUSTAL W alignment of DNA

sequences. Phylogeny tree was grouped into two main groups, I and II (Fig. 8). Members in each group shared high identity scores ranging from 92% to 100%. Nucleotide substitution rate varies from 0 to 9.3.

Fig. 8. A dendrogram depicting relationships among **Y** genome STS marker sequences of *Pseudoroegneria* (8389, 8376, and 3257) and *Hordeum* species (8140, 8281, 8299, 8279) in relation to those from polyploidy *Elymus rectisetus* (JC51= BV679236) and *E. longiaristatus* (5992). Numbers in parentheses are the number of identical sequences represented by the displayed sequence at the end of each branch.

The original RAPD marker sequence (JC51) was aligned in the same subgroup (I-1) with five clones from *P. spicata* (PI 232134). Clones from *Elymus longiaristatus* were scattered in subgroups I-2 and I-3, where three clones from *P. spicata* and seven clones from *P. libanotica* were located. Four clones from *Pseudoroegneria spicata* formed the subgroups I-4. In subgroup I-5, five clones from *P. ferganensis*, had 100% identity. Group II was clearly defined by *Hordeum* species. Sequences from both *Hordeum murinum* (**Xu**) and *H. marinum* (**Xa**) were intermingled in this group; thus, they were more distantly related to the **Y** genome sequences from *E. longiaristatus* than those from

any *Pseudoroegneria* species. There was little difference among sequences from the two *Hordeum* species, *H. murinum* and *marinum*, which are grouped together.

DISCUSSION

The present study aimed at searching for the origin of the **Y** genome in *Elymus* species. Two hundred twenty six accessions of perennial Triticeae, mainly in *Elymus, Hordeum*, and *Pseudoroegneria*, were screened for the **Y** genome specific STS marker, B14 F1/R1-269bp. A total of forty three accessions of *Elymus* species were tested. They were classified into three groups: (1) 23 accessions known to have the **Y** genome in their genomic constitution, (2) 10 accessions known not to have the **Y** genome, and (3) 10 accessions with unknown or questionable genome combinations.

In group I, only one out of 23 failed to detect the **Y** genome by the STS marker. *Elymus praeruptus,* T-217, previously classified as a tetraploid with **StY** genomes**,** did not amplify the three **St** and the one **Y** genome STS markers. This result is surprising, because the species was previously known to possess the **StY** genomes. The STS markers allowed the detection of **Y** genome in *Elymus* species with a 95% confidence (one out of 23 failure rate). There are two possible explanations for this negative result (1) the used plant material was not the said species, or (2) the priming sites for the STS marker sequence had base changes during the evolutionary process of the species.

In group II, accessions known not to carry the **Y** genome, one out of ten accessions tested positive for the **Y** genome marker and nine other accessions were confirmed to lack the **Y** genome marker. *Elymus fibrosus* (PI 547320), previously classified as containing the **StH** genome, was tested positive for the **Y** genome marker. However, with further scrutiny based on the intensity of the marker fragment in Fig. 7; this positive result could be attributed to the presence of **Y** genome sequence in the **St**

genome rather than the presence of a true present-day **Y** genome. Therefore, *E. fibrosus* should still be regarded as an **StH** genome tetraploid.

In group III species, for which the genomic formula is unknown, we found that five out of ten species possess the **St** and **Y** genomes. They are: *Elymus curvatus* PI 531579, *E. angustigglumis* PI 531639, *E. laxiflorus* KJ 278, *E. kunlunshanicus* CPI 11942, and *E. praecaespitosus* X-93019. However, the following accessions of *Elymus* species lack the **Y** genome but possess the **St** genome markers- *E. interruptus* PI 531617, *E. scabriglumis* PI 202147, *E. villosus* PI 531703, *E. scabrifolius* PI 531544 and E. *angustigglumis* PI 531639.

The presence of the **St** genome in the *Elymus* and *Pseudoroegneria* species was tested with three STS markers, B04F/R-341bp, D15F/R-498bp, and N05F/R-520bp. None of these three markers could singly detect the presence of **St** genome. Even the use of all three markers failed to detect the **St** genome in three *Elymus* species (*E. fibrosus, E. tsukushiensis,* and *E. praeruptus*; Table 10) and two accessions of *P. libanotica* (PI 401325 and PI 401331 (Table 12). Comparing to these **St** genome STS markers, the **Y** genome marker is more reliable. This may be due to the longer evolutionary passage experienced by the **St** genome than the more recently evolved **Y** genome.

Testing the **Y** genome marker in *Hordeum* with the primer pair B14F1/R1 demonstrated that there is no diploid *Hordeum* species that could be the source of **Y** genome. The accessions of *Hordeum* that tested positive for the **Y** genome marker are all tetraploids: *H. murinum* PI 422469, *H. murinum* PI 206686, *H. murinum* ssp *leporinum* NGB 90353.2, *H. murinum* sp *murinum* NGB 6530*, H. murinum ssp murinum* NGB 6870.2*, H. murinum ssp leporinum* NGB 90251.2*, H. marinum* NGB 90606.2*, H.*

marinum ssp gussoneanum NGB 90249.2*, H. murinum ssp leporinum* NGB 90350.1*, H. murinum ssp leporinum* NGB 90352.1, *H. murinum ssp leporinum* NGB 90353.2, and *H. murinum ssp leporinum* NGB 90599.2 (Table 11). Our study focused on the diploid donor of the **Y** genome. These results suggested that **Xa** and **Xu** genomes in *Hordeum* are not candidate donors of the **Y** genome to the *Elymus* species.

The **Y** genome must have higher copy numbers of the repetitive STS marker (lanes 5 - 8 of Fig. 7) than all other genomes. The copy numbers of the **Y** genome marker in *Hordeum* are lower so that the bands are very faint compared to those in *Pseudoroegneria*.

The B14F1/R1-269bp STS marker has been found to be useful in searching for the **Y** genome donor in some of the *Pseudoroegneria* species. Our results confirmed that *Pseudoroegneria libanotica* (PI 401326), *P. spicata* (PI 232134), and *P. ferganensis* (T-219) are all diploids possessing the **Y** genome marker; thus, are potential donors of the **Y** genome. The **St** genome STS markers B04F/R-341bp, D15F/R-498bp and N05F/R-520bp were used to ascertain that these species have the **St** genome. Both **St** and **Y** genome STS markers were present in the three above-mentioned accessions of *Pseudoroegneria* diploids, making them the prime candidate donors of the **Y** genome to the **StY** genome *Elymus* species. Our results added additional evidence in supporting the hypothesis that the **St** and **Y** genomes may have originated from the same ancestor (Yen et al. 2005; Liu et al. 2006).

To further elucidate the relationships among **Y** genome markers in *Elymus*, *Pseudoroegneria* and *Hordeum* species, the amplified B14F1/R1-269 STS markers from suspected **Y** genome donor species were cloned, sequenced and aligned. Twelve clones

were isolated from *Pseudoroegneria spicata* (**St**), five from *Pseudoroegneria ferganensis* (**St**), seven from *Pseudoroegneria libanotica* (**St**), thirty nine from *Hordeum murinum* (**Xu**), seven from *Hordeum marinum* (**Xa**), and eight from the positive control *Elymus longiaristatus* (**StY**). These sequences were compared and aligned to determine their similarities to the **Y** genome's random amplified polymorphic DNA (RAPD) marker, JC51 (GenBank accession BV679236).

The phylogenic tree was based on the CLUSTAL W alignment of DNA sequences after identical sequences within a species were consolidated (Fig. 8). The phylogeny tree separated into two main groups, I & II. Each group was formed by high sequence identity; scores ranged from 92% to 100%. Nucleotide substitution varied from 0-9.3.

Y genome specific sequences amplified from *Pseudoroegneria* species showed a high level of similarity to JC51. Three distinct sequences from *P. spicata* accession were placed in the first group (Group I). One sequence of *P. spicata* is closely related to JC51 (Subgroup I-1). Two sequences of *E. longiaristatus* and one sequence from *P. spicata* are in subgroup I-2. One sequence each from *E. longiaristatus* and *P. libanotica* are located in subgroup I-3. In the subgroups I-4, and I-5, there is only one sequence each from *P. spicata* and *P. ferganensis*, respectively (Fig. 8).

Based on these results, it is clear that *Pseudoroegneria spicata* contains sequences that are most closely related to the **Y** genome STS marker in both *E. rectisetus* (**StWY**) and *E. longiaristatus* (**StY**). Therefore, *Pseudoroegneria spicata* is the prime candidate as donor of the **Y** genome to the *Elymus* species containing **StY, StPY, StWY** or **StHY** genome constitution.

The second group (II) in the phylogeny tree contains sequences from **Xu** and **Xa** genomes of *Hordeum murinum* and *H. marinum*, respectively. In contrast with McMillan and Sun's (2004) results, we found a clear separation between the **StY**, **XaXa** and **XuXu** genomes (Fig. 8). Based on both the copy number (Fig. 7) and sequence homology (Fig. 8), it is clear that the **Y** genome STS sequences in *Hordeum* species are more distantly related to that in present-day **Y** genome than those in the three *Pseudoroegneria* species identified in this study.

Stebbins (1975) stated that the ancestors of *Pseudoroegneria spicata* and *Hordeum* migrated from Asia to North America, hybridized and gave rise to some of the North America polyploids, and then later migrated to South America. The present study identified *P. spicata* as the prime source of **Y** genome in polyploid *Elymus* species having **StY, StPY, StWY** or **StHY** genome constitution. Then, these *Elymus* species must have been evolved before the migration of an ancestral species of *Pseudoroegneria spicata* from Asia to North America.

Generally, the evolution of plants is a very complicated process. Lu (1993c) reported that it is difficult to make a complicated evolutionary process fit a man-made taxonomic classification system. However, discovery of the origin of **Y** genome will contribute to our understanding of evolutionary process of *Elymu*s species that have this genome. It can be speculated that the **Y** genome marker was either eliminated or remained unamplified during speciation for many *Pseudoroegneria* species. On the other hand, a species with an **St** genome having the **Y** marker sequence hybridized with one having an **St** genome without the **Y** marker sequence. Rapid amplification of the repetitive marker sequence followed the hybridization and polyploidization events. This

amplification enriched the marker sequence in one of the two **St** genomes, leading to genome differentiation and allopolyploidy. The **St** genome with enriched **Y** marker sequence became the **Y** genome in the newly formed **StY** genome tetraploid species. Then, this **StY** species hybridized with species having **H, P** or **W** genome, resulting in hexaploid *Elymus* species with **StHY, StPY**, or **StWY**, respectively.

CONCLUSION

The **St** genome of *Pseudoroegneria* is the most important component in the genomic constitution of the *Elymus* genus at present. Based on Dewey's genomic classification system, the **St** genome is present in all *Elymus* species. However, the genomic constitution of approximately 40% of all *Elymus* species is still unknown and some have questionable genomic formulas. Using a **Y** genome specific STS marker, we obtained evidence supporting the hypothesis that the **Y** genome in some *Elymus* species shared a progenitor genome with the **St** genome. The STS markers allowed the detection of **Y** genome in *Elymus* species with at least a 95% confidence level (1 out of 23 failure rate). The STS marker for the **Y** genome is useful to analyze all polyploid *Triticeae* species whose genome constitutions are still unknown.

Three out of 43 accessions of *Pseudoroegneria* tested, were positive for the **Y** genome marker. Chromosome counts were carried out for these three accessions and were confirmed to be diploids (2n = 14). The DNA sequences of this **Y** genome marker in these three *Pseudoroegneria* species are more similar to those obtained from *Elymus* species having the **Y** genome than those from *Hordeum marinum* and *H. murinum*, which have the **Xa** and **Xu** genome, respectively. Thus, specific accessions of *Pseudoroegneria spicata* (PI 232134), *P. libanotica* (PI 401326), and *P. ferganensis* (T-219) are being suggested as donors of the **Y** genome to *Elymus* species having the **StY, StHY, StPY,** or **StWY** genome constitution. Their genome symbol could be designated as $\mathbf{St^Y}$. If *P. spicata* were the source of **Y** genome, polyploidization event forming the $\mathbf{StSt^Y}$ tetraploid

species that eventually evolved to **StY** species should have occurred before the migration of an ancestral species of *P. spicata* from Asia to North America.

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APPENDIX

Media formulation

LB Agar (per liter)

10 g of NaCl

10 g of tryptone

5 g of yeast extract

20 g of agar

Add deionized H_2O to final volume of 1 liter

Adjust pH to 7.0 with 5N NaOH

Autoclave

Pour into Petri dishes (25 ml/100 mm plates)

LB-Ampicillin Agar (per liter)

1 liter of LB agar, autoclaved

Cool to 55ºC

Add 10 ml of 10 mg/ml filter-sterilized ampicilin

Pour into Petri dishes (25 ml/100 mm plate)

2% X-gal (per 10 ml)

0.2 g of 5-bromo-4-chloro-3-indly-ß-D-galactopyranoside (X-Gal)

10 ml of dimethylformamide (DMF)

Store @ -20ºC

Spread 40 µl per LB-agar plate

SOB Broth (per liter)

20.0 g of tryptone

5.0 g of yeast extract

0.5 g of NaCl

Add deionized water to final volume of 1 liter

Autoclave

Add the following filter-sterilized supplements prior to use

10.0 ml of M $MgCl_2$

10.0 ml of M $MgSO_4$

SOC Broth (per 100 ml)

2 ml of filter-sterilized 20 % (w/v) glucose or 1 ml of filter sterilized 2 M glucose
SOB medium (autoclaved) to final volume of 100 ml.

Working solution for DNeasy 96-well plate protocol

	Volume per sample	volume for 2 x 96 sample
Buffer AP1 (Preheated to 65 ºC)	400 µl	90 ml
RNase A (100 mg/ml)	1 µl	225 µl
Reagent DX	1 µl	225 µl

DEFINITIONS

Plasmid is a small, circular piece of DNA that is often found in bacteria. These innocuous molecular bacteria survive in the presence of an antibiotic, for example, due to the genes it carries.

Vector is generally the basic type of DNA molecule used to replicate your DNA, like plasmid.

Insert is a piece of DNA we have purposely put into another (vector) so that we can replicate it.