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Richard R. C. Wang
U.S. Department of Agriculture

B. Shaun Bushman
U.S. Department of Agriculture

S. R. Larson
U.S. Department of Agriculture

I. W. Mott
U.S. Department of Agriculture

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Development of genomic tools for wheatgrass and wildrye improvement

R . R .-C . Wang , B . S . Bushman , S . R . Larson , I . W . Mott
USDA-ARS , Forage & Range Research Laboratory , Logan , UT 84322-6300 , USA , Richard .Wang@ars .usda .gov

Key words : bacterial artificial chromosome (BAC) ,expressed sequence tag (EST) ,mapping ,quantitative trait loci (QTL) , simple sequence repeat (SSR)

Introduction Wheatgrass and wildrye grasses are some of the most important grasses in the temperate regions of the world (Asay and Jensen , 1996a , 1996b) . Cultivars of wheatgrass and wildrye grasses are mainly used as forage crops on rangeland and pastures in North America . Their drought and/or salinity tolerance make them suitable plant materials for revegetation and land reclamation . As members of the *Triticeae* tribe , they are related to and have been hybridized with cultivated cereal crops including wheat (*Triticum* spp .) , barley (*Hordeum* spp .) , and rye (*Secale cereale* L .) as genetic sources for pest (disease and insect) resistance , salinity tolerance , and other traits .

Materials and methods To facilitate breeding of wheatgrass and wildrye grasses , we have developed expressed sequence tag (EST) libraries for bluebunch wheatgrass (*Pseudoroegneria spicata*) , *Elymus* , and *Leymus* as well as bacterial artificial chromosome (BAC) libraries for *Leymus* in cooperation with two collaborating universities (Bushman et al . 2008 ; Larson et al . 2007) . SSR markers in the *Triticeae* libraries were developed , tested for amplification in a suite of species , and used for genetic mapping on interspecific mapping populations (Wu et al . , 2003) . Overgo primers for screening BAC libraries were designed and tested . BAC clones containing target gene were identified , partially sequenced and mapped .

Results and discussion Three EST libraries have been constructed using various tissues/organs and treatments of *P . spicata* , *L . cinerius* × *L . triticoides* , and *E . wawawaiensis* × *E . lanceolatus* (Table 1) . We are using the EST library at this time for SSR and STS marker development , comparative genomics , expression studies and functional genomics . Two BAC libraries for the *L . cinereus*× *L . triticoides* hybrid with two restriction enzymes , *Bam*HI and *Mbo*I consist of 405 ,888 clones arrayed in 1 , 057 384-well microplates and have an average insert size of 150 .5 kb . The combined libraries are equivalent to approximately 6 . 1 × haploid genomes . The BAC libraries had been screened with 25 Overgo primer sets covering 23 genes , for which about 160 positive BAC clones were identified . BACs containing the orthologous *Lax* rice gene were mapped on group 3 chromosomes of the **Ns** and **Xm** genomes in the allotetraploid *Leymus* hybrid that are homoeologous to rice chromosome 1 . Selected BAC clones will be sequenced , aligned , and used for physical mapping . These genomic resources will lead to the development of molecular markers (SSR , STS , and SNP) to assist breeding efforts on wheatgrass and wildrye species .

Table 1 *EST libraries constructed and used .*

Library	Clones	%	BLAST hits Unigenes	Markers tested	Informative	Mapped
Pseudoroegneria	13 ,777	93	8 ,780	408	101	47
Leymus	14 ,393	91	11 ,281	1 ,656	741	200
Elymus	8 ,758	91	7 ,212	809	107	56

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