Analysis of *Bromus inermis* populations using Amplified Fragment Length Polymorphism markers to identify duplicate accessions

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Introduction The temperate grass germplasm collection maintained at the USDA, ARS Western Regional Plant Introduction Station (WRPIS) in Pullman, Washington, consists of more than 18,000 accessions. Passport and collection data suggest that some of these accessions are duplicates, and their maintenance unnecessarily drains limited resources. The objective of this study was to use Amplified Fragment Length Polymorphism (AFLP) marker analysis on 4 populations of smooth bromegrass, *Bromus inermis* Leyss. subsp. *inermis* `Manchar´, a cross-pollinated perennial grass, to determine if the genetic variation among them was significant. If not, then maintaining separate populations would be unnecessary.

Materials and methods Total nucleic acids were extracted from new-leaf tissue of 24 plants from each population using the MagneSil®[1] Kit by Promega and the concentration was adjusted to 250g/:l. Restriction enzyme digestion, ligation of adapter sequences, preliminary amplification, and selective amplification was performed using AFLP kits from Life Technologies. Selective amplification was performed using primers labeled with WELLRED® dyes for analysis on the Beckman CEQ8000 capillary electrophoresis apparatus. Two primer pairs were analyzed providing a total of 780 polymorphic markers whose polymorphic information content (PIC) ranged from 0.02 – 0.50. Total and within population variances (PIC values) were calculated using the formula:

$$1 - \Sigma p_{ij}^2$$

where *p* is the frequency of the *i*th allele at the *j*th locus. Identification of markers was performed automatically on the CEQ8000 with ancillary software and exported to a spreadsheet where allele frequencies and variances were calculated. PIC values are measures of genetic variance within populations (Wright, 1951; Nei, 1973, Hamrick and Godt, 1989, Culley *et al*., 2002). PIC values were calculated over all plants analyzed (total variance) and for each of the populations, and these values were used in the equation:

$$G_{ST} = 1-(H_S/H_T)$$

where H_S is the mean PIC value of each of the populations and H_T is the PIC value totaled over all individuals (combined from all populations). G_{ST} is defined as the proportion of genetic diversity that resides among populations (Nei, 1973). G_{ST} was calculated using the methods of both Nei (1973) and Hamrick and Godt (HG) (1989). The difference in the two methods is that the HG method averages G_{ST} values over all loci, while the Nei (1973) method calculates G_{ST} by first averaging H_T and H_S over all loci.

Results The methods produced G_{ST} values of 0.068(Nei) and 0.071(HG). Using the average of the PIC values obtained at each locus for each population as variances within populations, and the proportion of the total variance calculated using G_{ST} as variance among populations, an F-test (3 and 92df for among and within population variances, respectively) revealed no significant differences among populations. (F = 0.35).

Conclusions The results indicate that the 4 populations are very similar and it is not necessary to maintain them separately. Germplasm managers at the WRPIS will maintain one of the populations for distribution and the other populations will be inactivated.

References

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