'Breeding for Success: Diversity in Action' C.F. Mercer (ed).
Proceedings of the 13th Australasian Plant Breeding Conference,
Christchurch, New Zealand 18-21 April 2006. pp. 548-551.

The Noble Foundation hardinggrass (*Phalaris aquatica*) breeding program

Andy Hopkins, Malay Saha, and Lili Zhou
Noble Foundation, Inc., 2510 Sam Noble Parkway, Ardmore, OK, USA

Abstract. Hardinggrass has the potential to provide grazing during the fall to spring months in the south central USA. Here we describe a breeding program focused on developing improved hardinggrass cultivars for this region. More than 300 accessions were evaluated for persistence under heavy grazing in Oklahoma. The most promising of these accessions were evaluated for genetic diversity using AFLP markers. Accessions clustered closely in agreement with geographic origins with populations from Morocco representing a potentially novel source of germplasm. Two distinct breeding populations were constructed using this information. Additional populations, constructed using recurrent selection for survival under heavy grazing, have shown significantly greater persistence than currently available cultivars including 'Grasslands Maru'. Research is underway to develop high throughput methods to profile alkaloid composition and concentration in hardinggrass. These methods will be applied to determine genotype and genotype x environment effects on alkaloid composition of elite breeding populations and commercial cultivars. Results of these evaluations, along with animal safety trials, will be critical in determining which populations to release as improved hardinggrass cultivars for the south central USA.

Introduction

Hardinggrass (*Phalaris aquatica*) is a cool season grass of Mediterranean origin. A number of cultivars have been developed and are used in Australia, New Zealand and Argentina. However, hardinggrass has not been widely used in the USA despite the development of improved cultivars such as AU Oasis (Pedersen *et al*. 1983), Perla (Adams *et al.* 1975), and Wintergreen. Lack of awareness by producers, concerns regarding alkaloid toxicity, establishment difficulties and the predominance of tall fescue (*Festuca arundinacea*), have all limited the use of hardinggrass in the USA.

The focus of our breeding program is to develop cultivars of cool season perennial grasses with improved persistence in the south central USA. Currently farmers and ranchers rely on hay feeding or cool season annuals such as wheat (*Triticum aestivum*) and annual ryegrass (*Lolium multiflorum*) to provide forage during the fall to spring seasons. Perennial cool season grasses would greatly improve the economics of livestock production in the region. However, current cultivars of cool season grasses generally do not persist well in the Southern Plains because of drought and heat stress (Malinowski *et al.* 2003, Hopkins 2005). Preliminary evaluations of a wide array of cool season grass germplasm indicated that hardinggrass had the potential to persist under heavy grazing pressure in Oklahoma and Texas, and as a result, a breeding program with this species was initiated. Here we describe our efforts to develop improved hardinggrass cultivars for the southern Great Plains of the USA.

Materials and Methods

In 1997, approximately 50 accessions of hardinggrass, obtained from the USDA National Plant Germplasm System, were established as spaced plants, on approximately 0.6 m centers, in Ardmore, OK. Accessions were replicated twice where sufficient number of seedlings were available, ten to 20 plants per accession were evaluated.

Plants were grazed heavily in spring and summer, 1998, after which severe drought conditions ensued. Seed was harvested in spring 1999 from 31 surviving plants to form the population HG PI C1. A second cycle of selection for persistence under heavy grazing was subsequently conducted to develop HG PI C2. A similar approach was used to select for persistence in the cultivar 'Grasslands Maru', to form the populations MIP C1 and MIP C2. These populations, along with cultivar checks, were sown in small plot pure sward trials in Texas and Oklahoma and evaluated under heavy grazing.

A second group of more than 240 hardinggrass accessions, was evaluated in Ardmore, OK, using the same methods, during 1999-2001. The most promising accessions from this evaluation, along with Maru, HG PI C1, HG PI C2, MIP C1, and MIP C2, were evaluated for genetic variation within and among populations using AFLP markers as described by Mian *et al*. (2005).

Alkaloid profiles of several hardinggrass populations were generated using High Performance Thin Layer Chromatography (HPTLC) following a modified protocol of Anderton *et al*. (1999). Briefly, 0.2 g of freeze dried plant tissue was extracted with 1% HCL, washed with methanol in an SPE column, eluted with ammonia, and the resulting sample run on HPTLC plate. Alkaloid bands were then developed using a spray reagent. Standard solutions containing alkaloids of known concentrations were included in each HPTLC run. Plant alkaloids are identified by comparing band location and color with that of standards.

Results and Discussion

The HG PI C2 population has shown greater persistence than Maru, and selections derived from Maru, in a number of grazing tolerance trials (Table 1). Progress has not been made in selecting Maru for increased persistence, whereas estimates of progress from selection for HG PI C2 have not been possible to date. However, our breeding program is now focusing mostly on the HG PI population because of its greater persistence, with a third cycle of selection being completed in 2005.

A total of 17 plant introductions, as well as Maru and the four breeding populations mentioned above, were surveyed for molecular diversity using AFLP markers. Accessions clustered in close agreement with their geographical origins. Two accessions from Morocco formed a distinct cluster, with the remaining populations forming a second large cluster. A much greater proportion of genetic diversity existed within (74%) than among (26%) populations. Genetic diversity did not decrease within populations selected for persistence. As a consequence, we have constructed breeding populations based on the Moroccan accessions alone, and populations based on several of the other accessions that closely clustered. Selection is also being practised within several individual populations.

Information on alkaloid profile of our breeding populations will be needed prior to cultivar release. We are developing high throughput methods to determine alkaloid composition and concentration of hardinggrass. To date, we have been able to clearly separate 10 alkaloid standards using a modified HPTLC method, and have identified alkaloids in hardinggrass samples. Initial results indicate a wide range of alkaloid composition and concentration both within and among hardinggrass populations, with some of our breeding populations showing minimal amounts of toxic alkaloids such as dimethyltryptamine. Samples collected from three locations in OK and TX indicate minimal effect of environment on alkaloid profile of hardinggrass, although further samples are being generated to confirm this finding. Results of this research will be

used to identify populations for inclusion in animal safety trials. Eventually we hope to adapt Near Infrared Reflectance Spectroscopy to quantify one or more alkaloids in hardinggrass, and select plants with favourable profiles.

In summary, hardinggrass has potential for grazing systems in the southern Great Plains. Breeding work is continuing in order to develop persistent, productive cultivars with minimal risk of alkaloid toxicity.

Table 1. Mean stand percentages, and standard errors (SE), of various hardinggrass populations in Oklahoma and Texas.

Entry	0.5†	1.5	2.5
		Burneyville, OK (2001 trial)§¶	
Maru	100	5b	0b
MIP C1	100	0b	0b
MIP C2	100	0b	0b
HG PI C2	100	74a	32a
SE	5	12	2
		Burneyville, OK (2002 trial)	
Maru	96a	50b	42b
MIP C1	88b	14c	0c
MIP C2	98a	42bc	36b
HG PI C2	100a	100a	100a
SE	3	13	15
		Iowa Park, TX (2002 trial)	
Maru	81	54b	54b
MIP C1	80	26b	22b
MIP C2	93	37b	29b
HG PI C2	85	91a	93a
SE	7	16	18

†Age of stand in years.
§ 100 = full stand, 0 = complete loss of stand.
¶ For each trial, numbers followed by different letters indicate significant differences at P <0.10.

References

Adams TE, Love RM Jr, McLauchlan RS (1974) Registration of Perla koleagrass. Crop Science 14, 339.

Anderton,N, Cockrum PA, Colegate SM, Edgar JA, Flower K (1999) Assessment of potential for toxicity of *Phalaris* spp. via alkaloid content determination: *P. coerulescens*, a case example. *Phytochemical Analysis* 10,113-118.

Hopkins AA (2005) Grazing tolerance of cool-season grasses planted as seeded sward plots and spaced plants. *Crop Science* 45, 1559-1564.

Malinowski DP, Hopkins AA, Phinchak WE, Sij JW, Ansley RJ (2003) Productivity and survival of defoliated wheatgrasses in the Rolling Plains of Texas. *Agronomy Journal* 95, 614-626.

Mian MAR, Zwonitzer JC, Chen Y, Saha MC, Hopkins AA (2005) AFLP diversity within and among hardinggrass populations. *Crop Science* 45, 2591-2597.

Pedersen JF, Hoveland CS, Haaland RL, Berry CD (1983) Registration of AU Oasis phalaris. *Crop Science* 23, 597.