BREEDING NON-TOXIC PHALARIS (*PHALARIS AQUATICA* L.)

R.N. Oram[1] and J.P. Edlington[1]

[1] CSIRO Division of Plant Industry, GPO Box 1600, Canberra, ACT 2601

Summary. The known toxins in phalaris herbage are monomethylated and dimethylated tryptamines (TRYP) and tyramines (TYR), the β-carbolines (BC) derived from TRYP, and cyanogenic glycosides (HCN). The relative concentrations of these have been surveyed in 50 accessions and over 500 individual plants in the CSIRO germplasm collection, in breeding populations, and in cv. Holdfast. Preliminary data suggest that low concentrations of TRYP+BC and TYR result from homozygosity of incompletely recessive alleles at two unlinked loci. Fifty plants with low concentrations of TRYP, BC, and TYR and low - moderate levels of HCN, were found in 1994 and re-tested in 1995. Five individuals are being clonally propagated to produce seed for field testing of an experimental population (LoTox) at Western Australian and South Australian sites prone to phalaris poisoning. If the concentrations of TRYP+BC and of TYR are simply inherited, the low alleles at each locus will be backcrossed into all agronomic types - Australian, Sirosa/Holdfast, Sirocco and *Acid-tolerant*.

INTRODUCTION

In the 1960s, it was thought that the occasional toxicity of the herbage of *Phalaris aquatica* L. (phalaris) was due to dimethyltryptamine alkaloids (5). Therefore, the cultivar Sirolan was developed with only 5% as much of these alkaloids as cv. Australian (10). However, Sirolan herbage still proved to be toxic in some localities and years. A search for additional toxin(s), in which phalaris extracts were fractionated and tested for cardiac activity using a rat-heart muscle bioassay, led to the discovery of N-methyltyramine (MT), which has strong effects on heart function (1). N,N-dimethyltyramine had been found earlier (9), and selected against during the development of Sirolan, but this compound is much less toxic than MT. Furthermore, cyanogenic glycosides (HCN) appear to have caused some sudden death cases in sheep in NSW (3). Yet other cases of sudden death are caused by nitrate (7) or by an unknown agent which induces a polioencephalomalacia-like disease (2). Losses from the latter two causes can only be minimised by ensuring that hungry sheep are not grazed on phalaris-dominant pastures during the autumn danger period. Cobalt prophylaxis by slow-release ruminal pellets or pasture sprays will reduce the losses from phalaris staggers, which is caused by the dimethyltryptamines and β-carbolines (BC) (2). Because no information is available on the extent of genetic variability in the concentrations of BC, MT or HCN in phalaris herbage, chemical assays were used to search for variability successively in a germplasm collection, in breeding populations and in a recent cultivar. This paper reports the results and discusses their implications.

MATERIALS AND METHODS

Forty-five accessions of phalaris from the Mediterranean and Middle East regions, 12 phalaris cultivars, two accessions of *Phalaris arundinacea* and one of *P. coerulescens* were transplanted into the field at Ginninderra Experiment Station, ACT, at 1?1 m spacings during September 1992. Each entry occupied a five-plant row in each of four replicates arranged in a randomised complete block design. Plant material above 30 mm height was removed in late summer, 1993, and the plots were fertilised with 100 kg/ha each of superphosphate (9.1% P, 11.5% S) and ammonium nitrate (35% N). During winter, moderately short growth on the plants was sampled by cutting to 0.5 cm height. A 20 g sub-sample of live material, made up of four g from each of the five plants in a plot, was cut into 1 cm lengths and immersed in 100 ml 0.1M HCl. These samples were kept at 3^0 C and swirled periodically for three days before filtering the leachate. The alkaloids were adsorbed onto a strong cation exchange cartridge, eluted off with 6M ammonia in ethanol and separated on silica gel thin layer chromatograms, following the methods of Anderton *et al*. (1). The colour intensity of each spot was rated on a 0-6 scale on which standards equivalent to 1.79 mg of alkaloid per kg fresh weight were rated 4. A small set of accessions, primarily those low in TRYP, BC and TYR, was sampled again in autumn, 1994, and also assayed for HCN by a picrate paper test. The intensity of the brick-red colour developed by the reaction of the picrate with HCN was scored on a 0-4 scale in which 0 = no change and 4 = white clover control. The white clover HCN level probably varied between runs, but comparisons were made only between plants within runs.

Two other spaced plant trials, managed in the same way as the accession trial, were also sampled during winter, 1993. The first contained 104 half-sib families from a *broadly-based breeding population* (BBSP) and six cultivars grown in 8-plant rows replicated twice; the trial was planted in 1987. Three plants in each of 85 half-sib families and four cultivars in each replicate were sampled in the BBSP trial and herbage extracts were made in the same way as before. The same plants in this trial also were tested for HCN. The second trial, planted in 1993, contained a new cultivar code-named BP 92, and a similar number of cv. Holdfast plants. Of these, 69 Holdfast plants and 35 BP 92 plants were sampled and analysed. Additionally, 172 potted plants, which had been selected in the previous year for grazing tolerance on acid soils by Dr R.A. Culvenor, were assayed for TRYP, BC and MT.

Table 1. Relative concentrations of total tryptamines plus carbolines, N-methyl- tyramine and cyanogenic glycosides in herbage of phalaris accessions.

			Mean score	
		(0=nil,	6=high)	(0=nil, 4=high)
Accession	Source	Tryptamines + carbolines	N-methyl tyramine	Cyanogenic glycosides
Low alkaloid	accessions			
CPI 15021	Turkey	0.0	0.0	2.5
15591	Israel	0.0	0.5	2.0
19264	Israel	0.0	0.5	2.0
19289	Algeria	0.0	0.0	1.5
19344	Portugal	0.0	0.0	2.0
98202	Jordan	0.0	0.5	2.0
High alkaloid	accessions			

40 others	Range:			
	Low	0.5	0.5	
	High	3.0	5.0	
Control	cultivars			
	Australian	2.0	1.5	2.5
	Holdfast	0.0	1.0	2.5
	Sirolan	0.0	4.0	3.0
	Sirocco	0.0	5.0	3.0
	l.s.d.(P=0.05)	N.S.	2.5	1.0

RESULTS AND DISCUSSION

The concentrations of both TRYP and TYR alkaloids varied from low to high among the accessions (Table 1). The accessions with no detectable alkaloids came from several parts of the Mediterranean homeland of the species. None was free of HCN. Of the control cultivars, Holdfast was the lowest in TRYP, BC and TYR, as noted previously by Anderton *et al*. (1), but it was moderately high in HCN. Holdfast may be the safest cultivar available at present, but even so, one farmer has reported sheep losses on it (4).

Some of the low toxin accessions were used as parents of the broadly-based breeding population, and this population in turn contained low toxin individuals. The distribution of plants with varying total levels of TRYP and BC possibly was trimodal (Fig. 1), suggesting the segregation of two co-dominant alleles, L and H, at one locus. The observed numbers of the putative LL:LH:HH genotypes were 73:128:64, which fits the numbers expected for a random mating population at the Hardy-Weinberg equilibrium with the frequency of the L allele of 0.511 ($\chi^2 = 0.355$, P = 0.8-0.9). Similarly, the N-methyltyramine distribution was at least bimodal, suggesting the segregation of a dominant and a recessive allele at one locus. The frequency of the recessive allele, presumed to give a low concentration when homozygous, was 0.514. In this model, the alleles at the two loci segregated independently from each other ($\chi^2 = 2.07$, P = 0.1-0.2).

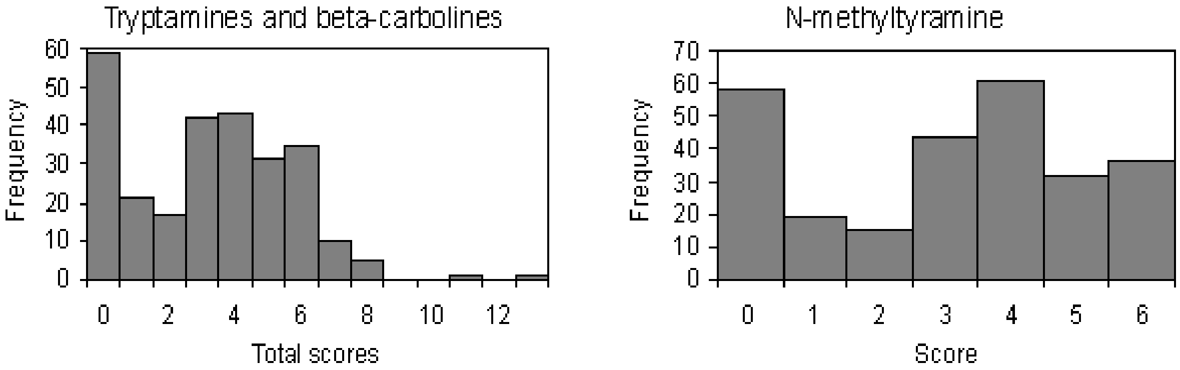

Figure 1. Frequency of plants in the broadly-based breeding population with various scores for tryptamines and β-carbolines and for N-methyltyramine (n=265).

If simple inheritance of low TRYP+BC and of MT is confirmed in the progeny of low x low, low x intermediate and low x high crosses now being assayed, the low alleles at both loci could be backcrossed into any cultivar or breeding population.

The cultivar Holdfast, which has some ancestry in common with the broadly-based breeding population, had a higher proportion of the double low phenotype (14 out of 69 plants tested for TRYP+BC and MT). Some additional double lows were found in four current breeding populations being selected for tolerance to acid soils and heavy grazing. A total of 50 double low genotypes from the breeding populations and cv. Holdfast was assembled in the spring of 1994. These were retested in autumn, 1995, under a higher level of N nutrition, which is known to increase the concentrations of TRYP (8), and probably has similar effects on the other nitrogenous toxins. Only five plants remained low in TRYP+BC and MT, and also were moderately low in HCN. These will be clonally propagated and intercrossed to produce seed of an experimental population for testing near Naracoorte, SA, and Esperance, WA, where phalaris poisoning is common.

Reduction in TRYP+BC and MT should reduce the incidence of phalaris staggers and of the form of sudden death which is precipitated by a nervous stress, such as being rounded up and driven by dogs (2). However, there will still be sudden deaths from nitrate poisoning and from the polioencephalomalacia-like disease (PEM). This latter disease typically kills a high proportion, e.g. up to 14 %, of sheep in an undisturbed flock during the first 12-48 hours of grazing on a fresh, toxic, phalaris dominant pasture (3). Affected survivors show vacuolation of certain brain cells at post-mortem examination, so this symptom could be used to identify districts where PEM is the common form of sudden death. In such districts, and anywhere that nitrate accumulates to high levels in the herbage, future cultivars of phalaris with low TRYP+BC, MT and HCN would not be less toxic than earlier ones, and there, cautious grazing management will remain the only way to avoid stock losses. The reduction of the toxins may render phalaris more susceptible to damage by corbies, grass grubs, crickets and wingless grasshoppers, which cause more injury to grasses such as perennial ryegrass and cocksfoot which lack the phalaris toxins. Some direct evidence on this point was provided by Lovett and Hoult, who showed that N,N-dimethyltyramine and gramine, which are closely related MT and TRYP, respectively, had inhibitory effects on the growth of armyworm larvae (6). However, it remains to be determined whether the concentrations of toxins can be reduced to a point where they deter insects but don’t harm ruminants. If so, the development of cultivars with low levels of the known toxins should reduce stock losses over the whole range of regions to which phalaris is adapted, and should encourage many more growers to benefit from the high productivity and sustainability of phalaris-based pastures.

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