Gramine: the occurrence of a self defence chemical in barley, Hordeum Vulgare L.

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Summary. Past work (4,9,11) suggests that the barley plant produces chemicals that may be active in defending the plant against interference from other species. This potential for self-defence, if well-developed, could reduce reliance on synthetic biocides, a significant cause of concern in the context of sustainability of agricultural systems. Although not a trait shared by all barley material, the ability to produce one of these metabolites, gramine, may be a heritable characteristic (1). We surveyed a range of barley material to determine whether this ability may have been lost in the process of domestication. Our results tend to support this hypothesis.

Introduction

A major concern of modern agriculture is sustainability and for some time now considerable energy has been expended in the search for pesticides which are highly active biologically but lack negative ecological or environmental effects. A complementary approach might be to apply the increasing knowledge of the mechanisms by which organisms interfere or interact with each other through the medium of natural biocides.

Overland (8) recognised the reputation of barley as a 'smother crop' and demonstrated that exudates of living roots were effective inhibitors of certain weeds. She tentatively identified the compounds involved as the alkaloids gramine and/or hordenine, the phytotoxic potential of which has subsequently been confirmed (4). Gram ine has also been implicated in self-defence by barley against aphids and bacteria (9,11). In reed canary grass, *P halaris arundinacea,* high gramine content reduces palatability and weight gain in sheep and cattle (6).

In reed canary grass the ability to produce gramine is controlled by one recessive gene (7). Consistent differences in gramine production between barley cultivars indicate that other genetically controlled factors within the plant may operate to modify gramine production (1). These results were interpreted to indicate that high gramine production was linked with cultivars developed to withstand hot climates (1). It is possible, however, that under breeding programmes which emphasise quantity and quality of yield, the ability to produce gramine has been lost or severely modified (for example, in reed canary grass low alkaloid content was correlated with higher crude protein content) (6).

With the exception of cvv. Prior and Proctor (1), nothing is known of the ability of Australian-grown cultivars to produce gramine, and very little about other types of barley. In this paper we report on a survey undertaken to investigate the gramine contents of a range of barley material including ancestral or weedy types, landraces, Middle Eastern lines and cultivars grown in Australia, past and present.

Methods

With the exception of five *Hordeum spontaneum* lines and the barley landraces, genetically pure seed was obtained for each line investigated from the Australian Winter Cereals Collection, Tamworth, NSW. All seed was multiplied at Arm idale prior to use. In total, 43 lines were investigated comprising six lines of *H. spontaneum,* four lines of *H. agriocrithon,* eight landraces, nine lines originating in the Middle East and 16 cultivars grown in Australia.

Plant material

Fifty seed samples, replicated three times, were grown in sand to the stage of one fully-expanded leaf, the seedlings separated into root and shoot and the material stored in a freezer until analysis. To confirm genetic control, two experiments were run under different conditions. In Experiment 1, 30 lines were grown in a growth chamber, 12 h day, 8-18?C, 430 W/m^2. In Experiment 2, all 43 lines were grown outside; mean daylength 10 h, mean temperature range 10-23?C, mean max. incident light 890 W/m^2, mean total incident light 6030 W/m2/d. Dormancy problems were encountered with all but one of the landraces and these were all resown at a later date along with two other lines for comparison (10 lines in all). Data for these lines are, therefore, not strictly comparable with those for other lines.

Extraction and purification

Samples (1.5 g) of frozen leaf tissue were extracted, purified and concentrated, following the method described by us elsewhere (3) with the following amendment: by using 0.05 M KH2P0, pH 2.5/isopropanol (60:40) as the final eluent in the purification protocol gramine recovery was found to be more reliable (90-95%). Gramine recovery was monitored by subjecting two 10 ml aliquots to the purification and concentration protocols with each batch of samples processed.

Identification and quantification of gramine

Samples (10 pl) of the concentrated extracts were injected into the HPLC and eluted isocratically with the mobile phase 0.025 M KH_2PO_4 pH 7.15/acetonitrile (60:40) (3). Identification was by retention time relative to, and by co-elution of representative samples with authentic gramine. Quantification was by mean peak area of dual injections for each sample relative to standard authentic gramine and calculated using a Waters 745 Data Module. Where appropriate, samples containing large amounts of gramine were diluted 10 or 20 times.

Results and discussion

Our data (Fig. 1) confirm the main findings of Hanson *et al. (1),* namely, that not all lines produce significant amounts of gramine and that amongst those that do, the ranking from high to moderate production does not change materially when barley plants are grown under different conditions. This implies genetic control. Of the Australian-grown lines, nine did not produce gramine; however, the detection of small but significant quantities of gramine in four of these (cvv. Forest, Windich, Schooner and Galleon) in Experiment 2, suggests that these cultivars may possess a gene(s) for gramine production but that the expression of this gene is almost totally suppressed, only the more favourable conditions of Experiment 2 making the production of trace amounts possible. The consistently greater gramine content of plants in Experiment 2 compared to Experiment 1 implies that there may be competition between metabolic pathways and that under the better conditions of temperature and incident light of Experiment 2, more substrate was available for diversion into the synthesis of the secondary metabolite. Linear regression of Experiment 2 on Experiment 1, for all 30 lines common to both experiments and for the 18 gramine producing lines, is presented in Figure 1. Comparison of the values for r^2 (r^2 = 0.908 and 0.730, respectively) indicates a looser genetic control of gramine production amongst the lines producing significant amounts of gramine.

All the *H. spontaneum* and *H. agriocrithon* lines and the landraces produced high levels of gramine. This is consistent with the results of Hanson *et al.* (1) who found high gramine levels in the *H. spontaneum* lines they investigated. The levels of gramine detected in the *H. agriocrithon* lines may have been underestimated: these are six-rowed barleys and, to a greater or lesser degree depending on the line, the lateral seeds did not germinate until later than the central seeds. Gramine production was found by Hanson *et al.* (2) to peak in seven day-old seedlings and it is possible that not all the *H. agriocrithon* seedlings would have reached this stage. The data for the landraces (Table 1) are probably also underestimated since the gramine content of the lines grown with them and also with the main group of Experiment 2 is of the order of 20% lower.

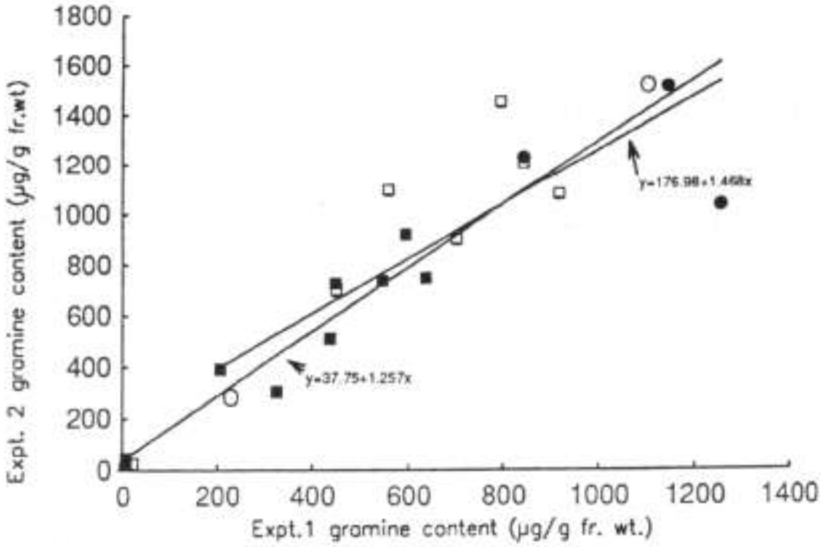

Figure 1. Gramine content of 30 barley lines.
(● = *H. spontaneum*, ○ = *H. agriocrithon*, □ = Middle Eastern lines,
■ = Australian lines.)

Although not a large sample these data suggest that, in barleys which have not been subject to conscious genetic manipulation by man, the ability to produce gramine is the norm. Analysis of variance for unequal replicates (10) applied to the five groups, namely *H. spontaneum, H. agriocrithon,* landraces, Middle Eastern and Australian, showed that there were significant differences between the means for each the *H. spontaneum* group and the Middle Eastern and Australian groups (Table 1).

Table 1. Mean gramine content (mg/g fresh weight) of five different groups of barley lines.

Group	No. entries	Gramine content
H. spontaneum	6	1410
H. agriocrithon	4	848
Landraces	8	830
Middle Eastern	9	706
Australian	16	274
l.s.d. (P=0.05) 606		

The trend detected towards decreasing gramine production as the degree of domestication increases and, indeed, for the ability to be lost almost entirely in some highly bred lines, supports the hypothesis that breeding for standard agronomic traits may lead to loss of self-defence capability in crop plants such as barley (5). The results of this survey indicate that the ability to produce gramine may be the norm in barley but that as a consequence of breeding for greater production, this ability may be modified or lost, along with a potential for self-defence.

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