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BARLEY PROTEINASE INHIBITORS: A POSSIBLE ROLE IN GRASSHOPPER CONTROL?*

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Abstract—Young growing barley seedlings contain trypsin and chymotrypsin proteinase inhibitors in their leaf juices. The amount of chymotrypsin inhibitor varies greatly while trypsin inhibitor content is nearly the same in all varieties tested. Compana (CI5438) barley has much more total inhibitor than does Trebi (CI 936), Titan (CI 7055), Horn (CI 926), Hiproly (CI 3947) and Hiproly Normal (CI 4362). The distribution of inhibitors in different barley varieties correlates with the severity of grasshopper damage observed by other workers. Barley leaves could not be induced to accumulate proteinase inhibitors after excision and incubation, wounding, or absorption of crude "proteinase inhibitor inducing factor" preparations. Grasshopper damage experiments as related to proteinase inhibitor should be done in the field using yield as the correlative factor.

INTRODUCTION

One of the worst pests of grain crops is the grasshopper and much work has been done to develop plants which are resistant to grasshopper damage. In the case of barley, experiments during the late thirties and early forties by Litzenberger [1] indicated that different varieties of barley in Montana were variably susceptible to grasshopper damage. Specifically it was observed that Compana and Glacier varieties were less susceptible than Horn or Trebi varieties. Compana was outstanding in that it was twice as resistant as Glacier.

Wide varietal differences in the amount of grasshopper damage among barley varieties were also noted at the Dominion Experimental Station, Swift Current, Saskatchewan, in the early forties. Field studies there by McBean and Platt [2] from 1944 to 1947 indicated that there were consistent differences in the amount of grasshopper damage suffered by different barley varieties. No single factor responsible for resistance was determined. However, the later maturing varieties and those high in protein suffered greatest damage by the grasshoppers. In the work reported here we have attempted to investigate a biochemical factor as a possible contributor to the variable susceptibility of barley to grasshopper damage. This involved the study of protein proteinase inhibitors which occur naturally in barley.

Green and Ryan [3] reported that wounding of the leaves of potato or tomato plants by adult Colorado potato beetles, or their larvae, induces a rapid accumulation of proteinase inhibitor throughout the tissues that are exposed to air. The same effect was observed after a mechanical-crushing wound was applied. No accumulation of inhibitor was observed in the plant when leaves were detached by a single, sharp razor blade cut through the petiole. Furthermore, the transport of a proteinase inhibitor inducing factor (PIIF) out of the damaged leaves takes place rapidly after the wound is inflicted and the levels of proteinase inhibitor, in both damaged and adjacent leaves, rises strikingly within a few hr. Green and Ryan [4] suggested that PIIF is a signal from the wounded tissues that may be part of a primitive immune response that produces proteinase inhibitors directed toward the proteolytic digestive enzymes of invading insects, or toward extracellular proteinases of invading microorganisms. Presumably the increasing presence of digestive inhibitors would increasingly deter the attacking insect.

McFarland and Ryan [5] established that PIIF activity is generally distributed throughout the plant kingdom. Extracts of barley leaves were strongly active in inducing proteinase inhibitor accumulation in tomatoes. Induction of inhibitors in barley by barley PIIF was not reported.

Kirsi and Mikola [6] have shown that barley (var. Pirkka) contains at least three different types of proteinase inhibitors. Tzeng and Hapner (unpublished observations) have isolated and characterized trypsin and chymotrypsin inhibitors from waxy Compana and Hiproly barley. We felt that varietal differences in the residual levels or in the induction and accumulation of barley proteinase inhibitors was possibly related to differential susceptibility to grasshopper damage. Several experiments were designed to test this hypothesis.

RESULTS AND DISCUSSION

Different varieties of barley were first assayed to determine the amount of inhibitor present in the resting, undisturbed plants. Table 1 shows the distribution of inhibitors in the six varieties tested. The amount of

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Table 1. Trypsin and chymotrypsin inhibitor activity of barley plant extracts with and without an incubation period prior to analysis. The results are expressed as μmol active trypsin or chymotrypsin inhibited per ml of extract

Variety	No incubation		Incubation	
	Chymotrypsin	Trypsin	Chymotrypsin	Trypsin
Compana	24	17	26	18
Trebi	6	17	8	17
Hi Proly Normal	9	14	10	14
Titan	4	14	6	14
Hi Proly	2	16	5	14
Horn	2	14	4	12

chymotrypsin inhibitor is quite variable but is highest in Compana and lowest in Hiproly and Horn. Nearly three times as much chymotrypsin inhibitor was present in Compana as Hiproly Normal, the next highest.

Trypsin inhibitor levels in the different varieties does not vary greatly. Compana and Trebi have the largest amount while the other varieties have slightly less. The total amount of trypsin and chymotrypsin inhibitor in Compana is nearly twice that of any other variety tested. Horn contains the least inhibitor. Compana also appears unique in that it contains more chymotrypsin than trypsin inhibitor. All other varieties contain much more trypsin than chymotrypsin inhibitor.

Although the significance of these relationships is not clear, certain comparisons may be drawn with previous work concerning grasshopper damage to barley. In all cases Litzenberger [1] found Compana (and Glacier) to be clearly more resistant than Horn and Trebi, and Horn was most susceptible to grasshoppers in three of four trials. McBean and Platt [2] also found Horn to be relatively susceptible, with Trebi and Titan being intermediate and Glacier being relatively resistant. These trends, in general, correspond with the relative amounts of proteinase inhibitors present in the young plants. Those plants containing the most inhibitor were least susceptible to damage and vice versa. These workers pointed out that other factors such as protein content, stage of maturity, and environmental factors may also be correlated with grasshopper damage.

Experiments designed to induce larger amounts of proteinase inhibitor in barley were generally negative but meaningful. Table 1 shows that an incubation period following excision had little effect on the inhibitor content. Very slight increases or no increase was observed when the leaves were excised from the plant and incubated in light and water for 28 hr prior to assay. Under similar conditions detached potato leaflets accumulate large amounts of proteinase inhibitor [8]. It has been shown however that cautious excision of the leaves may not result in inhibitor accumulation, even though the plant has the capability to do so [9]. Barley leaves or whole plants could not be induced to accumulate proteinase inhibitor either by wounding or by imbibing tomato leaf juice. When the plants were mechanically injured and then incubated, results essentially identical to those in Table 1 were seen. Barley lacked any PIIF-like response. Crude PIIF preparations from barley or tomato had no effect on inhibitor levels when fed to excised barley leaves. Ryan [9] has shown that tomatoes accumulate several hundred μg inhibitor per ml juice when stimulated with PIIF.

McFarland [5] earlier showed that barley extracts (containing barley PIIF) induces tomatoes to accumulate inhibitor. Apparently the barley varieties tested do not have the capacity to respond to tissue injury by accumulating proteinase inhibitors as has been demonstrated in the case of potato and tomato plants. It would follow that the extent of any physiological role which the inhibitors may play would depend upon the normal physiological concentration of the inhibitors within the plant tissue.

Attempts to demonstrate grasshopper susceptibility or induction of proteinase inhibitor as a result of grasshopper damage were inconclusive. It is well known that grasshoppers will consume barley plants without regard to variety if the infestation is sufficiently severe. In these experiments the insects seemed to select the healthy and vigorous plants irrespective of the variety. Additionally the young age of the plants resulted in leaves being entirely clipped from the plant which in turn did not leave sufficient material for reliable assay. In short, we believe field experiments on a much larger scale are necessary to produce adequate materials for assessment of damage (by yield differences) and for adequate proteinase inhibitor assays. Correlations among proteinase inhibitors and yields, as influenced by grasshoppers, would likely be a more meaningful overall measure than would be seedling susceptibility alone.

EXPERIMENTAL

Inhibitor assay. Barley plant juices were assayed for trypsin and chymotrypsin inhibitors titrametrically using a pHstat [7]. Substrates used were *N*-tosyl-L-arginine Me ester and *N*-acetyl-L-tyrosine Et ester, respectively. Inhibitor content is expressed as μmol of active trypsin or chymotrypsin inhibited per ml of leaf juice.

Varieties tested. Barley varieties used in this study included Horn, Trebi, Titan, Compana, Hiproly and Hiproly Normal. Plants were grown from seeds in sterilized soil under summer greenhouse conditions. When 15–20 cm high, the plants were ground in a cold mortar and pestle. The resultant juice was clarified by filtration through cheese cloth and centrifugation (3×10^4 *g*, 30 min). An aliquot was then assayed for inhibitor activity.

Inhibitor induction. Whole plants (15–20 cm) or leaves were excised with a razor blade and incubated in H_2O in a humid chamber at 31° and 100 lx fluorescent illumination. After 24–28 hr the plants were assayed for inhibitor content. Some plant leaves were injured by compressing one or two places with a pair of pliers prior to incubation. Other excised plants were 'fed' extracts of barley and tomato leaves prior to incubation.

Crude PIIF preparation. Tomato or barley plants were first steamed for 5–10 min and then extracted as described in ref. [5]. The juice was clarified by centrifugation (2.3×10^4 *g*, 5 min) and labeled "crude PIIF".

Grasshopper studies. Five pans (45 cm × 30 cm) were planted with 10 plants of each of four varieties of barley:

Trebi, Horn, Titan and Compana. 5 other similar pans contained Horn, Compana, Hiproly and Hiproly Normal as the barley varieties. When the plants were 15–20 cm high, 4 pans from each set were placed in separate screened cages. The remaining 2 served as controls. Twenty grasshoppers, *Camnulla pellucida*, which had been captured in the field, were placed in each cage and observations recorded. In another expt, 25 plants of each variety were planted in each of 2 pans. When the plants were 15–20 cm high, one of the pans was placed in a cage with 5 grasshoppers. After a period, the damaged plants and the control plants of each variety were assayed for inhibitor content.

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