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TRYPSIN INHIBITOR ACTIVITIES IN THE WILD PROGENITOR OF BARLEY

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Key Word Index—*Hordeum vulgare* subsp. *spontaneum*; Gramineae; wild barley; trypsin inhibitor.

Abstract—Trypsin inhibitor activities of dormant kernels were determined in 22 strains of wild barley originating in Southwest Asia and raised in Finland. All of the strains showed trypsin inhibitor activity. The inhibitor levels in the endosperms varied much more than those in the embryos. On a dry weight basis, the wild barley endosperms had, on average, 70% (ranging from 8 to 137%) of the inhibitor activity of the control (cv Pirkka). The embryonic inhibitor activities were comparable to those of cv. Pirkka. The inhibitor activities in endosperms and embryos varied independently of each other, and did not significantly correlate with the contents of water-soluble proteins.

INTRODUCTION

The trypsin inhibitor present in the endosperms of barley is chemically a rather well-characterized protein [1–4] which represents a quantitatively important fraction of water-soluble proteins of barley grains [5]. The inhibitor level varies greatly between different cultivars [6]. We were interested in the possible existence of trypsin inhibitors in the kernels of the wild progenitor of barley, *Hordeum vulgare* subsp. *spontaneum* (C. Koch) Thellung, because it was impossible to predict if the domestification and cultivation of barley have changed the trypsin inhibitor contents of the kernels. Moreover, their grains are exceptionally rich in proteins and have a strong post-harvest dormancy [7]. In order to see if the high protein content of *spontaneum* barleys and their deep dormancy are associated with trypsin inhibitors, a sample of 22 strains of wild barley was assayed for trypsin inhibitor activity. Since the trypsin inhibitors are different in the embryo and endosperm of barley [5, 8], the two organs were studied separately.

RESULTS AND DISCUSSION

All of the endosperm and embryo extracts contained trypsin inhibitor activity (Table 1). No trypsin inhibitors were present in the husks. The endosperms showed a wide variation in inhibitor levels between different strains, and the levels did not correlate with the contents of water-soluble proteins ($r = -0.035$, not significant). The highest degree of activity (2.28 units/g) was found in a strain originating from the Coast of Galilee, Israel, and the lowest one (0.14 units/g) in a strain from an unknown region in Israel. On average, the trypsin inhibitor activities, on a dry weight basis, were lower than those found in the Pirkka control and in different cultivars of barley [6]. This set of the 22 different cultivars studied by Kirsi [6] had a mean of 1.87 ± 0.097 units/g, ranging from 1.2 to 3.0 units/g. The present wild barley sample was significantly different ($t = 4.51$, $P < 0.001$), the mean being 1.15 ± 0.119 units/g. The results indicate that even within a small geographical region (Judean Foothills) the endospermal trypsin inhibitor content can be highly variable in wild barley populations. Likewise, hordeins, the main storage proteins of barley, are highly variable in wild barley populations. Hordein polymorphism has recently been found to be associated with differences in soil type and topography over quite short distances [9].

Trypsin inhibitors were assayed from embryos in only a portion of the material. The embryos of wild barley showed distinctly higher activities than the endosperms (Table 1), as did the embryos of cultivated barleys [8], and the inhibitor levels were quite similar to those detected in cultivated barleys [8] when different extraction procedures are taken into account (defatting with acetone was not used in the present study). The variation in inhibitor levels in the embryos of wild barleys was smaller than that in the endosperms, and the endospermal and embryonal inhibitor levels were totally independent of each other.

In their native habitats, the dispersed seeds of *spontaneum* barleys are stored for months in the ground [10]. It would be possible that during the underground storage trypsin inhibitors may in part determine the digestibility of the kernels to seed-consuming animals and micro-organisms. However, the results of this study show that the trypsin inhibitor levels in the kernels of wild barley are not higher than those in the kernels of cultivated barley. Also, the specific activities of the endospermal inhibitors are lower than in the control (Table 1). This seems to suggest that the high protein content and the deep post-harvest dormancy are not associated with a high trypsin inhibitor content.

Since we found so much variation in the endospermal trypsin inhibitor content, it would be useful to investigate the genetic basis of the variation, and which environmental properties, if any, are associated with the different inhibitor levels in wild barleys. Such an evaluation might increase the knowledge of the significance of trypsin inhibitors to the plant.

EXPERIMENTAL

Plant material. The *spontaneum* strains used comprised a part of the material described by Ahokas [7]. Twenty accessions had

Table 1. Trypsin inhibitor activities and water-soluble protein content in the endosperms and embryos of dehusked wild barleys originating in different localities

		Endosperms			Embryos		
			Inhibitor activity			Inhibitor activity	
Country	Region	Soluble protein (mg/g dry wt)	(U/g dry wt*)	(U/g sol. protein)	Soluble protein (mg/g dry wt)	(U/g dry wt*)	(U/g sol. protein)
Afghanistan	Unknown	11	0.75	68	44	4.36	98
Iran	Unknown	15	1.39	93	34	5.37	156
Israel	Judean Foothills	13	0.71	55	47	5.67	121
Israel	Judean Foothills	15	0.42	28	46	7.43	160
Israel	Judean Foothills	15	1.09	73	46	5.50	120
Israel	Judean Foothills	11	0.53	48	n.d.†	n.d.	
Israel	Judean Foothills	12	2.14	178	n.d.	n.d.	
Israel	Judean Foothills	10	1.12	112	n.d.	n.d.	
Israel	Judean Foothills	10	1.17	117	n.d.	n.d.	
Israel	Upper Galilee	19	1.06	59	47	5.78	123
Israel	Upper Galilee	10	1.13	113	n.d.	n.d.	
Israel	Lower Galilee	14	1.31	94	n.d.	n.d.	
Israel	Upper Jordan Valley	13	2.26	174	n.d.	n.d.	
Israel	Coast of Galilee	13	2.28	175	n.d.	n.d.	
Israel	Coast of Yam Kinneret	12	1.21	101	n.d.	n.d.	
Israel	Mt. Carmel	11	0.56	51	n.d.	n.d.	
Israel	Sharon Plain	11	1.56	142	n.d.	n.d.	
Israel	Jerusalem Mountains	13	1.69	130	n.d.	n.d.	
Israel	Unknown	12	0.97	81	54	6.18	115
Israel	Unknown	13	0.14	11	49	4.97	100
Israel	Unknown	17	0.89	52	57	8.68	153
Israel	Unknown	14	0.89	64	46	7.20	153
Finland	Control, cv Pirkka	8	1.66	208	49	6.68	137
	Mean‡	12.9	1.15	91.8	47.0	6.11	130
	Standard deviation‡	2.31	0.57	47.2	6.09	1.30	24

*Mean of two to five determinations.

†n.d. = Not determined.

‡Cv Pirkka excluded.

Coefficients of correlation (cv Pirkka excluded): soluble protein vs inhibitor activity in endosperms, $r = -0.042$, not significant; soluble protein vs inhibitor activity in embryos, $r = 0.520$, not significant; endospermal vs embryonic inhibitor activity, $r = 0.005$, not significant.

their origin in Israel, one in Iran, and one in Afghanistan. The material was vernalized and raised in a homogenous experimental field in southern Finland as described elsewhere [7], along with the control cv Pirkka. All the grains were dehusked by hand, and the embryos were separated after incubation for 4–5 hr in a shallow layer of de-ionized H_2O. The endosperms and embryos were dried for at least 3 days at 20° and ground in a cyclone mill with a 0.4 mm screen.

Extraction and inhibitor assay. Trypsin inhibitors were extracted as described earlier [8], the extracts were separated by centrifugation (30 000 *g*, 30 min), and dialysed. The trypsin inhibitor assay [1] was based upon hydrolysis of benzoyl-D,L-arginine-*p*-nitroanilide.

Protein determination. The protein content of the dialysed extracts was determined by the Lowry method as described by Bailey [11].

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