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PEROXIDASE AND AMYLASE ACTIVITY IN DEVELOPING GRAINS OF TRITICALE, WHEAT AND RYE

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Abstract—Changes in the activities of peroxidase and amylase were measured during the development of grain of triticale, wheat and rye. Peroxidase and amylase activities were found to be higher in Triticale-1 which possesses highly shrivelled grains. A direct relationship between the degree to which the grain is shrivelled and the activity of peroxidase and enzymes was observed. During grain development, peroxidase and amylase activity per grain increased in Triticale-1, while it decreased in wheat, rye and well filled triticale grains.

INTRODUCTION

Analysis of proteins and starch characteristics has indicated that triticale, a man-made taxon derived from a cross between wheat and rye, simply inherits the two sets of genomes and exhibits characteristics that are intermediate between wheat and rye. [1–5] This new species has not become a commercial crop, due to grain shrivelling, which could be the result of genetic, physiological or biochemical factors. Since it has already been established that triticale normally shows higher protein content than wheat [6], a study of enzymes other than proteases might explain why grain shrivelling occurs. In this paper, an analysis of peroxidase and amylase in wheat, rye and triticales during grain maturation, is presented.

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RESULTS AND DISCUSSION

Triticale, wheat and rye kernels at different stages of development were assayed for peroxidase and amylase activities. The specific activity of peroxidase (Table 1)

Table 1. Peroxidase activity in developing kernels of triticale, wheat and rye

		1972–73				1973–74			
				Peroxidase activity				Peroxidase activity	
Plant	No. of days from anthesis	Fresh wt. of kernel (mg)	Protein/ kernel (mg)	per g fr. wt	per mg protein	Fresh wt. of kernel (mg)	Protein per kernel (mg)	per g fr. wt	per mg protein
Russian Rye	10	18	0.252	120	8.33	26	0.273	95	9.45
	17	34	0.378	66	5.93	38	0.447	71	6.04
	24	24	0.300	100	8.00	27	0.333	111	9.00
	31	23	0.347	127	8.42	23	0.294	124	9.49
Triticale-1	10	34	0.318	161	18.39	27	0.294	192	16.56
	17	58	0.391	163	23.97	48	0.396	182	21.82
	24	45	0.312	198	27.90	43	0.300	197	27.90
	31	39	0.399	232	21.41	37	0.335	223	24.86
M-1019 (Triticale)	10	25	0.303	190	15.37	29	0.343	185	15.62
	17	50	0.399	91	11.29	60	0.445	66	8.89
	24	41	0.407	109	10.85	48	0.430	73	8.14
	31	35	0.429	102	8.20	36	0.411	89	7.80
NP 404 (Wheat)	10	36	0.324	94	10.44	32	0.288	96	3.07
	17	72	0.531	66	8.95	67	0.537	60	4.02
	24	50	0.361	73	7.52	45	0.453	73	3.21
	31	43	0.450	58	5.48	41	0.459	60	2.46

Table 2. Amylase activity in developing kernels of triticale, wheat and rye

Culture	No of days from anthesis	Amylase activity 1972–73 Per g fr. wt	1972–73 Per mg protein	1973–74 Per g fr. wt	1973–74 Per mg protein
Russian Rye	10	1913	145	1736	152
	17	875	80	750	64
	24	900	75	616	52
	31	670	47	768	55
Triticale-1	10	1072	115	1490	171
	17	1054	124	1250	138
	24	1425	153	1500	156
	31	1844	181	1666	180
M-1019 (Triticale)	10	1843	158	1700	150
	17	821	80	877	85
	24	770	71	893	77
	31	721	58	814	65
NP 404 (Wheat)	10	1077	152	1318	158
	17	543	66	445	152
	24	548	60	455	43
	31	450	39	320	29

was highest in Triticale-1 which had maximum grain shrivelling, while M-1019, with less shrivelling had lesser enzyme activity compared to T-1. The enzyme activities in rye and wheat were lower than triticales at all stages of grain maturation. At maturity the activity in T-1 was two-fold higher compared to the activity in Russian Rye and four-fold compared to wheat grain. The activity in M-1019 was higher than wheat but lower than rye. Comparison of protein content of the kernel showed that wheat kernels had highest protein content, while T-1 had intermediate protein content. The enzyme activity, per g fresh weight or per mg protein, was highest in T-1. During kernel development peroxidase activity decreased in wheat and M-1019, on the other hand it increased in T-1.

Amylase activities in T-1, wheat and rye also showed similar differences during grain maturation (Table 2). The amylase activity increased during grain development in T-1, while it decreased in other materials. A similar trend was observed during two seasons, confirming that the changes associated in peroxidase and amylase are characteristic of T-1 and are related to grain shrivelling.

The levels of peroxidase and amylase activity per kernel are plotted against stage of maturation in Fig 1a and b, respectively; it is clear that the changes in the levels of these two enzymes are characteristic and are different. Peroxidase activity in wheat decreased drastically during maturation, while in rye it increased slowly. This is in contrast to the sharp increase in peroxidase activity in T-1, during early grain development and its stability during the maturation process (Fig. 1a). In triticale M-1019, which has relatively better developed kernels, the levels of enzyme activity resembles that of wheat. The amylase activity per kernel in wheat, rye and M-1019 decreased throughout the maturation process, whereas in T-1 the activity increased around 17 days post-anthesis and henceforth remained stable at that high level of activity (Fig. 1b). From the genomic nature of triticale, one would expect the level of peroxidase and

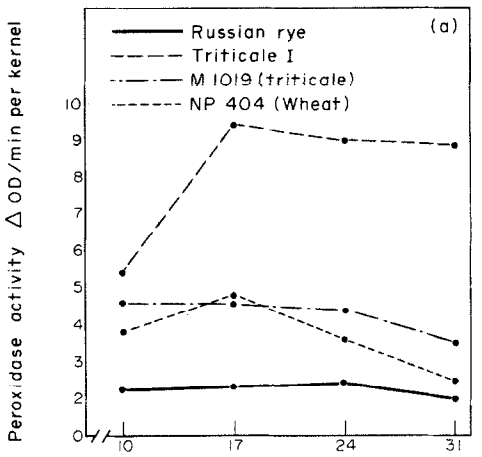

Fig. 1a. Peroxidase activity in triticale, wheat and rye at various stages of grain development.

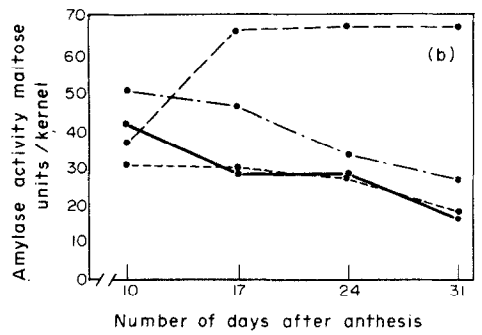

Fig. 1b. Amylase activity in triticale, wheat and rye at various stages of grain development.

amylase activities to be intermediate between wheat and rye. The deviation in the activity of enzymes suggests that there is a definite interaction between wheat and rye genomes in triticale.

In order to find biochemical basis for seed shrivelling in triticales, two enzymes, namely peroxidase and amylase, have been studied during the grain development. The amylase has been taken because it is a degradative enzyme and related to starch metabolism. Peroxidase was selected because of its role in growth and development. [7–8] The behaviour of peroxidase and amylase in triticale was entirely different from that observed in its parental species, suggesting an interaction of their genomes in the hybrid. The results indicate that a direct relationship may exist between the activities of these enzymes and seed shrivelling. The increase in amylase activity and its relation with shrivelling of seeds in triticale is in agreement with the finding of earlier workers [9]. Its lower activity in M-1019, a triticale with well filled seeds and higher activity in Triticale-1, a poorly developed kernel type, suggest a direct relationship between enzyme activity and the amount of seed shrivelling. This is further supported by a further result which showed lower peroxidase and amylase activities in two mutants with better developed kernels compared with the control material (97141). Peroxidase activities (ΔA/min/mg protein) were for the control and the two mutants 16.85, 9.2 and 10.05; amylase activities (maltose units/mg protein) 79, 44 and 39 respectively. The enzyme

activity in 97141 itself, which is intermediate between M-1019 and T-1 in kernel shrivelledness, is intermediate to the values found in M-1019 and T-1. Though the precise role of peroxidase is not known, it is possible that peroxidase and IAA oxidase are one and the same protein [10,11]. Accumulation of peroxidase in slow growing tissues and dwarf plants suggests a growth inhibiting activity of this enzyme. By the same analogy, it is likely that higher activity of peroxidase may also be responsible for grain shrivelling in triticales. Hence, both amylase and peroxidase may be important in determining shrivelling in triticales. This emphasises the need for triticale genotypes with low activity of peroxidase and amylase, in the improvement of triticales.

EXPERIMENTAL

Two triticale lines, viz. M-1019 and Triticale-1 (T-1), representing well developed and plump grain and highly shrivelled grain, respectively were selected and grown on IARI farm in the winters of 1972–73 and 1973–74. For comparison, a *duram* wheat N.P. 404 and Russian Rye (*Secale cereale*) were also grown. The soluble proteins were extracted from kernels at different stages of development by grinding of about 20 seeds in pestle and mortar in the presence of Tris–Cl buffer (pH 7.6; 1:3 w/v). The homogenate was centrifuged at 10000 rpm for 15 min for assaying peroxidase activity. For amylase assay the extraction was done in H_2O. All operations were carried out at 4° unless otherwise stated. Protein was estimated by the method of Lowry *et al.* [12]. Peroxidase activity was assayed with slight modification of the method used by Shannon *et al.* [13]. The activity has been expressed as change in absorbance per min per mg protein at 460 nm. The amylase activity was assayed according to the method of Bernfeld [14]. The enzyme activity has been expressed as maltose units per mg protein. The enzyme assays were conducted for two crop seasons i.e. during winters of 1972–73 and 1973–74. The values reported are an average of at least duplicates which agreed closely.

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