Theor. Appl. Genet. 55, 87-92 (1979)

THEORETICAL AND APPLIED GENETICS

Peroxidase Activities in Relation to Plant Height and Grain Weight in Bread Wheat (*Triticum aestivum* L.)

N.C. Singhal, S.L. Mehta and M.P. Singh
Division of Genetics, Indian Agricultural Research Institute, New Delhi (India)

Summary. The relationship of peroxidase activity with plant height and grain weight has been studied in seven different varieties of bread wheat belonging to diverse genotypes, and their F_1 crosses. The association between plant height and peroxidase activity was highly significant and negative. Based on the similarity index values of peroxidase isoenzymes, the seven wheat genotypes could be classified into two groups: the first group consisting of triple and quadruple dwarf varieties and the other of tall, single and double dwarf. A negative correlation between peroxidase activity and grain weight was also observed. However, the results of this study indicate a possibility of developing a dwarf plant type with low peroxidase activity and well filled grains.

Key words: Peroxidase activity – Plant height – Grain weight – *Triticum aestivum* L.

Introduction

Gel electrophoresis techniques have been used in understanding developmental processes and phylogenetic pathways in various crop plants. The level of peroxidase activity has been found to be variable in different genotypes. The activity is reported to be positively associated with reduction of plant height in pea (Müller 1969), tomato (Evans and Alldridge 1965) and triticale (Liang 1974). Additional experimental evidence pertaining to the role of isoenzymes in economic plants could lead to the basis of planned biochemical breeding for crop improvement. It is in this context that the present investigations were initiated: to find out the relationship of peroxidase activity to plant height and its correlation with grain weight in different varieties of bread wheat belonging to diverse genotypes.

Materials and Methods

Seven wheat varieties namely, 'HD1917', 'HD 1553', 'Kalyansona', 'Hira', 'Moti', 'Mex. C.B. 116', 'Olesen's dwarf' and their 21 F_1's were analysed for peroxidase activity. Peroxidase isoenzyme pattern was studied in parents.

Growing of Seedlings

Seeds sterilised with 0.1 per cent mercuric chloride for 1-2 minutes, were subsequently washed with distilled water and allowed to germinate on wet filter paper in sterilised petri dishes. Petri dishes were kept in illuminated germinating chambers at 25°C.

Extraction of Soluble Proteins for Peroxidase

For studying the peroxidase activity and isoenzyme pattern, coleoptiles from 72 hours imbibed seeds were hand-ground in 0.05 M Tris-Cl buffer (pH 7.6, 1:5 W/V) with a chilled pestle and mortar at 4°C. The cell paste suspension was centrifuged at 10,000 × g for 15 minutes. The supernatant obtained was used for peroxidase assays and for gel electrophoresis.

Protein Estimation

Protein was estimated by the method of Lowry et al. (1951) using folin phenol reagent and bovine serum albumin as the standard.

Electrophoresis

Polyacrylamide gel electrophoresis was used to separate peroxidase isoenzyme. The anionic system described by Ornstein (1964) and Davis (1964) was used. Samples containing 200-225 μg protein were layered above the spacer gel. The electrophoresis was run at 4°C at 3.0 mA current per gel collumn. After completion was run at indicated by the movement of the tracking dye (Bromo phenol blue) to the bottom, the gels were removed and stained for peroxidase isoenzymes.

0040-5752/79/0055/0087/$ 1.20

Detection of Peroxidase Isoenzymes

Gels were incubated at room temperature in following reaction mixture for half an hour.

0.5 per cent 0-dianisidine hydrochloride – 1 ml
0.6 M Sodium acetate buffer (pH 5.4) – 3 ml
water – 26 ml

The gels were then incubated in 0.1 M H_2O_2 until the visible bands were developed. Thereafter the gels were transferred to 7 per cent acetic acid solution. The gels were scanned in a Joyce-Loeble Chromoscan, using appropriate filters. Two independent extractions were made for all the material examined. For each sample duplicate runs were made. The Rf value of each band was calculated as indicated below:

$$Rf = \frac{\text{Distance of band from the top of gel}}{\text{Distance travelled by tracking dye}}$$

Similarity index: It was calculated according to the method suggested by Sheen (1972):

$$S = \frac{\text{Similarities}}{\text{Similarities + dissimilarities}} \times 100$$

Where similarity was taken as the number of pairs of similar bands on the basis of their Rf value in the two varieties. A dissimilarity was indicated by the number of different bands in both the entries.

Peroxidase Assay

Peroxidase was assayed according to the method of Shannon et al. (1966). The activity has been expressed as change in absorbance per minute per mg protein or per g fresh weight at 460 nm.

Results

Peroxidase Isoenzymes

The wheat varieties included in this study were selected on the basis of their distinct height differences. Peroxidase enzyme band patterns of seven varieties are presented in Table 1 and illustrated in Figure 1. A total of 8-12 bands were observed. Three bands with Rf 0.07, 0.21 and 0.59 were common to all seven varieties. The intensity of the band with Rf .07 was the same in all varieties, whereas bands with Rf 0.21 and 0.59 were less intense in 'Hira' and 'Olesen's dwarf' as compared to others.

Peroxidase isoenzyme at the running gel origin was present only in 'Hira' and 'Olesen's dwarf'. The band with Rf 0.03 was common in dwarf genotypes, 'Moti', 'Mex. C.B. 116' and 'Olesen's dwarf' and bands at Rf 0.41 and 0.77 were present only in 'Hira', 'Moti', 'Mex. C.B. 116' and 'Olesen's dwarf'. On the other hand, two bands with Rf 0.11 and 0.83 were consistently confined to the tall and medium tall varieties 'HD 1917', 'HD 1553' and 'Kalyansona'. The band with Rf 0.13 was observed only in 'Mex C.B. 116' and 'Olesen's dwarf', whereas the band with Rf 0.53 was present only in 'HD 1917'. The band at Rf 0.46 was present in the tallest variety, 'HD 1917', as well as in the dwarfest variety 'Olesen's dwarf'. Another band at Rf 0.17 was recorded in three varieties, having contrasting

Table 1. Peroxidase isoenzyme pattern

RF values	Intensity						
	HD 1917	HD 1553	Kalyansona	Hira	Moti	Mex C.B. 116	Olesen's dwarf
0.00				XX			XX
0.03					X	XX	X
0.07	XX	XX	XX	XX	XX	XX	XX
0.011	X	X	X				
0.13						X	X
0.17	X			X			X
0.21	XXX	XXX	XXX	XX	XXX	XXX	XX
0.25		X			XXX	XX	XX
0.29		X			XXX	XX	XX
0.35			X		X	X	
0.41				X	X	X	X
0.46	X						X
0.53	XX						
0.59	XX	XX	XX	X	XX	XX	X
0.67		X	X	X	X		
0.71	XX	XX	XX			X	X
0.73		XXX	XXX				
0.77				XXXX	XXXX	XXXX	XXXX
0.83	X	X	X				

X = Light intensity, XX = Medium, XXX = Dense, XXXX = Very dense

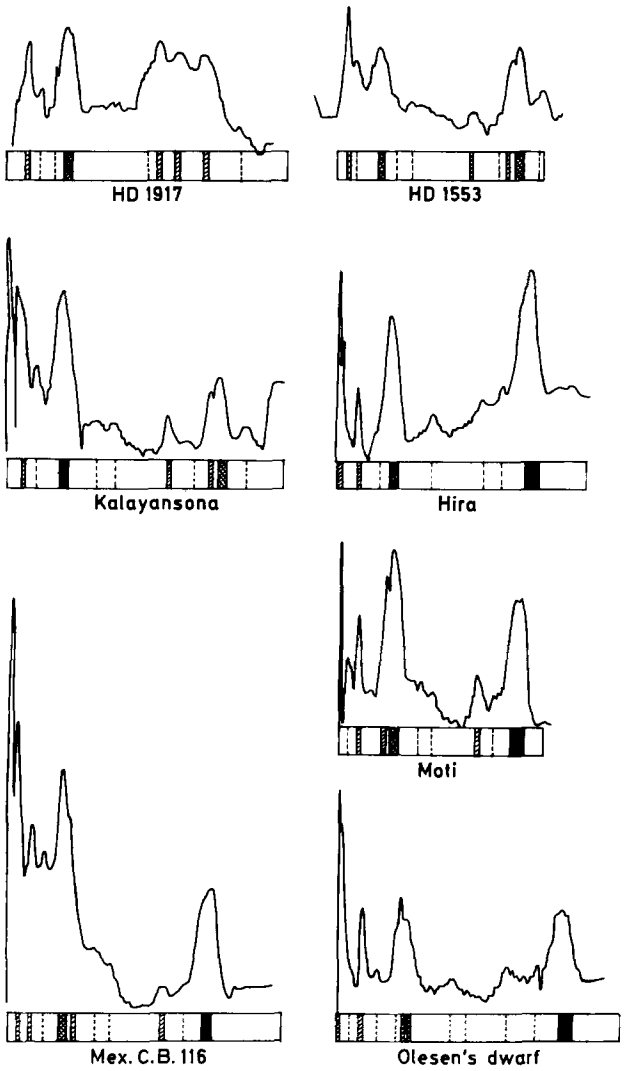

Fig. 1. Densitograms of isoenzymes of peroxidase in coleoptile of wheat varieties

height gradations, such as 'HD 1917' (126 cm) 'Hira' (78 cm) and 'Olesen's dwarf' (51 cm).

Similarity Index

Per cent similarity index in different combinations of seven wheat varieties, based on peroxidase isoenzyme band pattern, is summarised in Table 2. The data showed a maximum similarity between 'HD 1553' and 'Kalyansona' (81.8%) followed by a 72.7% similarity between 'Moti' and 'Mex C.B. 116' and a 69.2% similarity between 'Mex. C.B. 116' and 'Olesen's dwarf'. The similarity between 'HD 1917' and 'Moti' and 'Kalyansona' and 'Olesen's dwarf' was found to be less than 25%. The similarity index between the four dwarf varieties, 'Hira', 'Moti', 'Mex. C.B. 116' and 'Olesen's' dwarf was equal to, or greater than, 50% except between 'Hira' and 'Mex. C.B. 116'. These results indicate a greater homology among the dwarf varieties. Considerably reduced similarity index values of 'HD 1917', 'HD 1553' and 'Kalyansona' with 'Hira', 'Moti', 'Mex. C.B. 116' and 'Olesen's dwarf' indicated major differences between these two groups.

Table 2. Percentage similarities index of seven wheat varieties

	HD 1553	Kalyan-sona	Hira	Moti	Mex C.B. 116	Olesen's dwarf
HD 1917	46.1	46.1	30.7	20.0	26.7	40.0
HD 1553		81.8	30.7	35.7	33.3	29.4
Kalyansona			28.5	35.7	33.3	22.2
Hira				54.5	38.4	53.7
Moti					72.7	50.0
Mex C.B. 116						69.2

Table 3. Mean values on peroxidase activity, plant height and grain weight of seven wheat varieties

Varieties/ Crosses F_1	Plant height (cm)	100 grains weight (gm)	Peroxidase activity	
			Specific activity (OD/mg Protein)	OD/ G.F.W.
HD 1917	126.8	4.9	14.49	213.33
HD 1553	101.7	5.5	19.74	300.71
Kalyansona	96.3	3.3	19.61	301.49
Hira	78.2	3.2	17.93	303.25
Moti	74.0	3.5	16.82	294.74
Mex C.B. 116	57.5	3.3	20.71	312.08
Olesen's dwarf	51.4	2.2	22.56	318.49

Peroxidase Activity

The observations on mean plant height, 100-grain weight and peroxidase activities of seven wheat varieties are summarised in Table 3. The tallest variety 'HD 1917' had the lowest peroxidase activity both on per mg protein as well as per g fresh weight basis. On the other hand the shortest variety 'Olesen's dwarf' had the highest specific activity as well as the highest activity per g of fresh wt. of coleoptile.

Correlation Studies

The peroxidase activity has been estimated in parents and their F_1's by two different ways. A simple correlation has

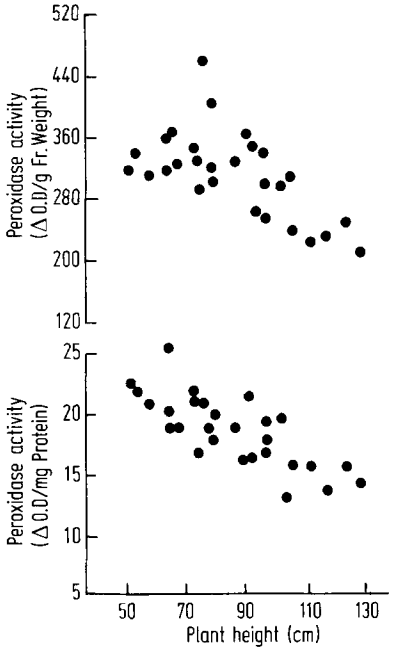

Fig. 2. The relationship of peroxidase activity with plant height

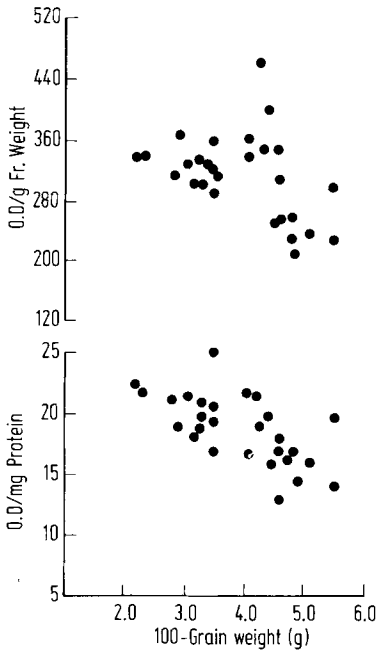

Fig. 3. The relationship of peroxidase activity with grain weight

Table 4. Relationship of peroxidase activity with plant height and grain weight

Characters	Correlation coefficient
Plant height *vs* O.D./mg protein	−0.77[a]
Plant height *vs* O.D./g.F.W.	−0.62[a]
Coleoptile length *vs* O.D./mg. protein	−0.76[a]
Coleoptile length *vs* O.D./g.F.W.	−0.70[a]
100 grain weight *vs* O.D./mg. protein	−0.64[a]
100 grain weight *vs* O.D./g.F.W.	−0.41[b]

[a] Significant at 1% level
[b] Significant at 5% level

been computed between the two estimates of peroxidase and other characters, including plant height and grain weight (Table 4). This has been done for pooled values of parents and F_1 s. The analysis clearly indicates that peroxidase activity is negatively correlated with plant height as well as with grain weight. The negative correlation in each case is highly significant.

Figures 2 and 3 show the relationship of peroxidase activity with plant height and grain weight. The grain weight and plant height have been ploted against the peroxidase activity. This has been done in relation to all values estimated for parents and F_1 s. It is obvious from the Figures that as the plant height and grain weight decreases the peroxidase activity increases. It is clear, however, that relationship is not very linear.

Discussion

Peroxidase is known to play an important role in the growth and development of plants. Therefore, in the present study the role of peroxidase in the growth of wheat was examined by relating plant height with the peroxidase activity in a set of seven varieties and their 21 crosses. The association between plant height and peroxidase activity was highly significant and negative. This indicated that reduction in peroxidase activity was associated with the tallness of the plant, and vice-versa. These results are in agreement with the observations of several other workers. While the exact biological function of the peroxidase versatile heme enzyme is not known, morphological roles are suggested by its action in producing (Riddle and Mazelis 1964) and inactivating auxin (Galston et al. 1953), in converting hydroxyphenylpropanes such as coniferyl alcohol to lignin-like materials (Siegel 1955) and in oxidizing such important metabolic compounds such as reduced nicotineamide adenine dinucleotide and its phosphate (William Ashman et al. 1959).

Overbeek (1935) observed that an equal quantity of growth substance was formed in dwarf as well as in normal plants but in the case of dwarfs more destruction took place during transport in the basal direction. As a result cell elongation was influenced. Relatively high catalase and peroxidase activity in dwarfs compared to normal plants has also been observed. Genetic dwarfism in peas (Brian and Hemmings 1955) and in maize (Phinney 1956) results from a block in the synthesis of gibberellic acid.

When certain dwarfs, especially single gene mutants, are treated with gibberellin to relieve dwarfism, their peroxidase activity falls precipitously as growth is promoted (McCune and Galston 1959). Conversely, when normal genotypes are stunted by the application of compounds which prevent gibberellin biosynthesis, the peroxidase activity rises markedly (Gasper and Lacoppe 1968). It is likely, therefore, that high peroxidase in dwarf wheat varieties act through auxin destruction. This is further supported by the observations that the two distinct wheat peroxidase isoenzymes effectively degrade IAA. Further peroxidase and IAA oxidase activities have been shown to be due to identical enzymes (Shin and Nakamura 1962, Fric 1971) in barley. The lesser peroxidase activity in taller wheat varieties would then result in the accumulation or conservation of auxin which ultimately enhance the growth.

Based on isoenzyme band patterns, the seven wheat genotypes could be broadly classified into two height groups. However, it was not possible to associate a particular band pattern with certain category of plant height. The first group comprising of triple and quadruple dwarf varieties such as 'Hira', 'Moti', 'Mex. C.B. 116' and 'Olesen's dwarf' had a greater similarity, while the other group comprising of tall, single and double dwarfs such as 'HD 1917', HD 1553' and 'Kalyan Sona' had greater homology.

This brings us to the question of the significance of higher and lower peroxidase activity in relation to growth and development. High peroxidase content is a symptom of an overall increase of dissimilative metabolism of the plant. The peroxidase activity was found to be negatively associated with grain weight. The negative correlation between grain weight and peroxidase activity suggests the involvement of peroxidase in the development of the grains. Such an association has been shown in relation to grain shrivelling in triticale (Rao et al. 1976). The results presented in this study indicate the involvement of peroxidase in determining height and grain development in wheat.

On the basis of our observations, let us examine the scope of increasing the grain weight in dwarf wheat varieties. The negative correlation reported between grain weight and peroxidase activity is not very large. There is considerable variation in the amount of peroxidase activity among the semidwarf wheats. It may be mentioned that the variety 'Moti' having triple dwarf height showed a relatively reduced activity of peroxidase which may partly account for its heavier kernel weight. On the other hand, the medium tall variety 'HD 1553', categorised as single dwarf, possessed relatively more peroxidase activity and heavier grain weight. There exists some deviation from the negative relationship due to certain specific characteristics, which in this case are associated with low number of grains per ear. It is also attributed to better assimilation ability of the genotype in spite of high peroxidase activities. If our results are viewed in light of general observations, it may be concluded that there seems to be a possibility of developing a dwarf plant type with low peroxidase activity and a well filled augmented number of grains.

Acknowledgement

The authors are grateful to Dr. B.R. Murty, the Project Director, Nuclear Research Laboratory for providing the research facilities.

Literature

Brian, P.W.; Hemmings, H.G.: The effect of gibberellic acid on shoot growth of pea seedlings. Physiol. Plant. **8**, 669-681 (1955)

Davis, B.J.: Disc electrophoresis. 2: Method and application of human serum proteins. Ann. N.Y. Acad. Sci. **121** 404-427 (1964)

Evans, J.J.; Alldridge, N.A.: The distribution of peroxidase in extreme dwarf and normal tomato (*Lycopersicon esculentum* Mill). Phytochemistry **4**, 499-503 (1965)

Fric, F.: Enzymes of indoleacetic acid degradation in barley leaves. Biologia (Bratialava) **26**, 677 (1971)

Galston, A.W.; Borner, J.; Baker, R.S.: Flavoprotein and peroxidase as components of the indole acetic acid oxidase system of peas. Arch. Biochem. Biopys. **42**, 456-470 (1953)

Gasper, T.; Lacoppe, J.: The effect of CCC and Amo-1618 on growth, Catalase, peroxidase and indole acetic acid oxidase activity of young barley seedling. Physiol. Plant. **21**, 1104-1109 (1968)

Liang, G.H.; Cunningham, B.A.; Lee, K.C.: Peroxidase activity in triticale. Ann. Wheat News Letter. **XX**, 129-130 (1974)

Lowry, O.H.; Rosenbrough, N.J.; Farr, A.L.; Randall, R.J.: Protein measurement with folin phenol reagent. J. Biol. Chem. **193**, 265-275 (1951)

McCune, D.C.; Galston, A.W.: Inverse effects of gibberellin on peroxidase activity and growth in dwarf strains of peas and corn. Plant Physiol. **34**, 416-418 (1959)

Müller, H.P.: Enzyme distribution in some radiation induced mutants of *Pisum sativum* with different internode length. Phytochemistry **8**, 1867-1871 (1969)

Overbeek, J. van: Growth hormone and the dwarf type of growth in corn. Proc. Nat. Acad. Sci. (US) **21**, 292-299 (1935)

Ornstein, L.: Disc. electrophoresis. 1: Background and theory. Ann. N.Y. Acad. Sci. **121**, 321-340 (1964)

Phinney, B.O. : Growth response of single gene dwarf mutants in maize to gibberellic acid. Proc. Nat. Acad. Sci. (Wash.) **42**, 185-188 (1956)

Rao, V.R.; Mehta, S.L.; Joshi, M.G.: Peroxidase and amylase activity in developing grains of triticale, wheat and rye. Phytochemistry **15**, 893-895 (1976)

Riddle, V.M.; Mazelis, M.: A role for peroxidase in biosynthesis of auxin. Nature **202**, 391-392 (1964)

Shannon, L.M.; Kay, E; Lew, J.Y. Peroxidase isoenzymes from horse raddish roots. 1. Isolation and physical properties. J. Biol. Chem. **241**, 2166 (1966)

Sheen, S.J.: Isoenzymic evidence bearing on the origin of *Nicotiona tabaccum* L. Evolution **26**, 143-154 (1972)

Shin, M.; Nakamura, W.: Indole – acetic acid oxidase activity of wheat peroxidase. J. Biochem. **52**, 444 (1962)
Siegel, S.M.: The biochemistry of lignin formation. Physiol. Plant. **8**, 20-32 (1955)
Williams-Ashwan, H.G.; Cassman, M.; Klavins, M.: Two enzymic mechanism for hydrogen transport by phenolic oestrogens. Nature **184**, 427-429 (1959)

Received January 22, 1979
Communicated by B.R. Murty

Dr. S.L. Mehta
Nuclear Research Laboratory
Indian Agricultural Research Institute
New Delhi-110012 (India)