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PEROXIDASE ACTIVITY AS A SCREENING PARAMETER FOR SALT STRESS IN *BRASSICA* SPECIES

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Abstract—The utility of peroxidase as an indicator of physiological stress in plants has been tested in 11 cultivars of *Brassica* in fresh water and saline media. No parallel was found between growth responses and changes in peroxidase activity irrespective of the basis for calculation of enzyme activity. The diversity of responses in peroxidase level among these cultivars is of interest in view of the function ascribed to peroxidase in hormonal regulation and lignification.

INTRODUCTION

Many biochemical changes are associated with the exposure of plants to harmful conditions. Among them increased peroxidase activity has been cited [1, 2] as an indicator of physiological stress. An elevated peroxidase level is induced by cold in alfalfa [3], conifers [4], peas [5], and tobacco [6]; drought in cotton [7] and wheat [8]; and hypoxia in bean and rye [9]. Salt stress in legumes has also been associated with elevated peroxidase levels [10, 11].

While screening various plant families, including the Cruciferae, for salt tolerant phenotypes [12], peroxidase activity was investigated as an indicator of salt-stress in young seedlings.

RESULTS AND DISCUSSION

Against the selective effects of half-strength sea water (*ca* 16000 ppm total dissolved solids), the poorest performance (fr. wt) was 9% of its control, and the best 54% (cultivars 1 and 11, Table 1). Between these two extremes are successive levels of performance in the saline medium, which have no consistent parallel in peroxidase activity. Finding no evidence here for elevation of peroxidase per unit wt of protein, the data were also calculated on the basis of activity per unit fr. wt of seedling. On this basis, the seedlings could be grouped as follows: <50%, cvs 8, 9; 50–75% cvs 2, 7; 75–100% cvs 1, 3, 4; 100–200% cv 5; 200% cvs 6, 10, 11. The 3 cultivars showing the best growth, 2, 9, 10, 11, are found at the extremes in this tabulation and those growing most poorly, 1, 2, and 3, are in middle groups. There is, thus, no parallel between growth and peroxidase responses irrespective of basis for calculation of enzyme activity.

The effect of varying salinity (as fraction of full strength sea water) upon Wong Bok (cv 10) confirms the complexity of growth-peroxidase relations (Fig. 1). The enzyme falls steadily in activity with rising salt content of the environment while growth as measured by fr. wt and shoot height pass through maxima before the onset of inhibition. Protein rises steadily, consistent with higher-than-control levels in Table 1. This in part reflects retardation of seed reserve utilization in saline media, but again cannot be correlated with variations in growth inhibition in the 11 cvs as a group. Wong Bok is a plant of particular interest because it can readily be grown to comparable harvest yields in our greenhouse on regimes ranging from fresh water to sea water dilutions of *ca* 12000 ppm total solids. There is reason to suppose that the 50% sea water regime may not be a stress condition in all of the

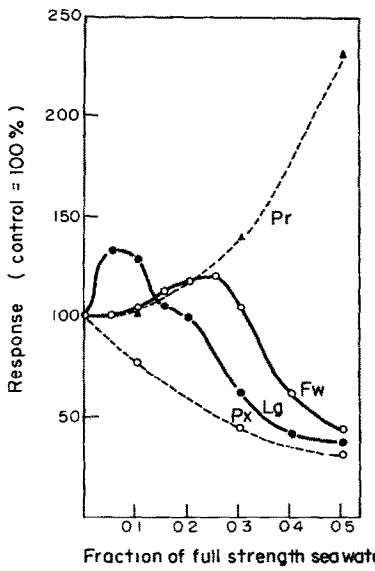

Fig. 1. Effect of variations in salinity upon growth and enzyme activity in cultivar 10, Wong Bok, after 7 days. Fw = fr. wt; Lg = shoot height; Pr = protein content and Px = peroxidase.

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cultivars used in this study. This question obviously does not apply to most of these populations but our experience with Wong Bok suggests that even relatively saline environments may be more regulatory than harmful and that adjustments to salinity may be a normal part of later development.

This view is consistent with the concept of phenotypic plasticity recently reported in experiment with the cultivation of barley strains in sea water [13], and with earlier evidence for physiological adjustment as a heritable character in plants [14].

These observations, although negative with respect to the practical objective, are nevertheless of interest because the diversity of response among such close-knit populations as the 6 cvs of *B. oleracea capitata* (head cabbage) must be considered in relation to the role commonly assigned to peroxidases in vascular development and auxin metabolism [15–18]. Conceivably, these physiological roles may be fulfilled in spite of greatly elevated or reduced peroxidase levels if the enzyme is not the limiting factor in these processes in the intact plant.

Table 1. Weight, protein and peroxidase levels of *Brassica* seedlings grown in fresh water and half-strength sea water

Cultivar	Fr. wt mg/25 seedlings	Lowry protein mg/g fresh	Peroxidase mg eq/g fr wt	Peroxidase mg eq/g protein
1. De Rapa Broccoli				
a. fresh	767	10	0.411	41.1
b. sea	69	50	0.310	6.2
c. b/a (%)	*9*	*500*	*75*	*15*
2 Ferry's Round Dutch Cabbage				
a. fresh	880	17	0.355	20.9
b. sea	86	30	0.246	8.2
c. b/a (%)	*10*	*177*	*69*	*39*
3. Red Dutch Cabbage				
a. fresh	850	15	0.158	10.5
b. sea	110	50	0.135	2.7
c. b/a (%)	*13*	*333*	*85*	*26*
4. Neptune Broccoli				
a. fresh	862	12	0.136	11.3
b. sea	127	23	0.122	5.3
c. b/a (%)	*15*	*192*	*90*	*47*
5. Pak Choi				
a. fresh	144	11	0.122	11.1
b. sea	31	38	0.190	5.0
c. b/a (%)	*22*	*346*	*156*	*45*
6. Savoy Chieftain Cabbage				
a. fresh	768	12	0.215	17.9
b. sea	200	38	0.555	14.6
c. b/a (%)	*26*	*317*	*258*	*82*
7. Copenhagen Market Cabbage				
a. fresh	948	12	0.188	15.6
b. sea	256	33	0.119	3.6
c. b/a (%)	*27*	*275*	*63*	*23*
8. Michihli				
a. fresh	770	12	0.475	39.6
b. sea	223	28	0.213	7.6
c. b/s (%)	*29*	*233*	*45*	*19*
9. Danish Ball Head Cabbage				
a. fresh	815	10	0.266	26.6
b. sea	259	35	0.077	2.2
c. b/a (%)	*32*	*350*	*29*	*8*
10. Wong Bok				
a. fresh	1070	6	0.233	38.8
b. sea	496	15	0.561	37.4
c. b/a (%)	*46*	*250*	*241*	*96*
11. Early Jersey Wakefield Cabbage				
a. fresh	536	2	0.075	37.3
b. sea	290	27	0.397	14.7
c. b/a (%)	*54*	*1350*	*529*	*39*

EXPERIMENTAL

Brassica cvs used in this study include the *B. oleracea capitata* group (cvs 2, 3, 6, 7, 9, 11); *B. oleracea botrytis* group (cvs 1, 4); *B pekinensis* group (cvs 8, 10), *B. chinensis*, (cv 5) (see Table 1).

In addition to shoot height and fr. wt after 7 days, protein was determined on seedling homogenates using the Lowry method (BSA as standard) [19] and peroxidase (EC 1.11.1.7) was assayed in aliquots of the same homogenates using 5 mM guaiacol and 5 mM H_2O_2 in 0.1 M Pi buffer pH 5.8 at 30°. Oxidation was followed at 470 nm. As a reference standard, Worthington crystalline peroxidase (R.Z. = 3.1, 3000 g/mg, batch HPODD 8AF) was used. Assay values were converted to mg-equivalents of reference enzyme preparation per unit wt of protein.

The presence of tissue NaCl in the homogenates had no effect on peroxidase activity. This was shown by addition of the salt up to a concn of 1 M. Previous work has shown that peroxidase activity is insensitive to the presence of NaCl even at saturation [20], and over the pH range 4.5–6.6

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