Phytochemistry, Vol. 21, No. 3, pp. 539–542, 1982.
Printed in Great Britain.

0031-9422/82/030539-04$03.00/0

REDUCTION IN PEROXIDASE IN *CUCUMIS, BRASSICA* AND OTHER SEEDLINGS CULTURED IN SALINE WATERS

S. M. SIEGEL,* JUNG CHEN,* WERNER KOTTENMEIER,* KATHERINE CLARK,* B. Z. SIEGEL† and HEALANI CHANG‡

*Department of Botany; †Pacific Biomedical Research Center; ‡School of Public Health, University of Hawaii at Manoa, Honolulu, HI 96822, U.S.A.

(*Received* 15 *May* 1981)

Key Word Index—*Cucumis* sp.; Cucurbitaceae; peroxidase; protein; salinity; stress.

Abstract—Among 11 *Brassica* and 10 *Cucumis* cultivars and one each of *Carica papaya* and *Zea mays* grown in various dilutions of seawater, there is no consistency of change in peroxidase activity when assayed on a fr. wt basis. In contrast, on a total protein basis, peroxidase activity was reduced by 50% or more in 17 of the 23 cultivars and by 18% or more in 21. The magnitude of peroxidase response is not related to the degree of fr. wt inhibition in saline media.

INTRODUCTION

In the course of a search for biochemical indices of salt stress, the utility of changes in peroxidase activity was tested in *Brassica* seedlings cultured in half-diluted seawater [1]. Elevation of peroxidase levels has been reported as a response to cold, drought, hypoxia and other stresses in a variety of plants [2–10] and has also been mentioned as a response to salt stress [11, 12]. In our study of *Brassica*, peroxidase levels on a fr. wt basis were increased in only four out of eleven cultivars, and reduced or unchanged in the remainder. Upon reconsideration of these data it was noted, however, that on a protein basis, peroxidase was suppressed in ten out of eleven cases. Further experimentation has revealed a similar pattern of peroxidase response in other plants as is reported here.

RESULTS AND DISCUSSION

Unlike *Brassica* cultivars, seeds of the cucumbers tested were almost incapable of germination in half-diluted seawater, but performed well (65–100%) at one-third dilution. These two saline media correspond to *ca* 16 000 ppm and 11 000 ppm total dissolved solids, respectively. The fr. wt of the plants varied by only about 30% among controls but approximately 2-fold in dilute seawater (Table 1). Lowry protein content was more variable among cultivars spanning a 3-fold range whether in fresh or saline media.

Peroxidase activities varied only 2-fold in both media on a fr. wt basis, but 3 to 4-fold when calculated per mg protein. In these experiments, all of the cucumber cultivars exhibited marked increases in protein per g fr. wt. Some of these increases simply reflect proportional reductions in fr. wt. Examples are cultivars 1, 2, 4, 9 and 10. In the remaining five cases, elevated protein content exceeds the expected reciprocity. Thus, cultivar 3 has a fr. wt ratio of 0.39 and might be expected to exhibit an apparent protein increase of 2.56 (1/0.39). Instead, the protein ratio is 7.00. The basis for differential responses among members of this close-knit group of cultivars is a matter for future investigation. It should be noted, however, that salinity-enhanced protein elevation is not restricted to one group of plants, having been noted in *Brassica pekinensis* (Wong Bok) a highly salt-tolerant crucifier [1]. Furthermore, such protein changes may take place at salinities that do not suppress growth and also on a dry wt basis [13].

To facilitate comparisons, both *Brassica* and *Cucumis* data have been arrayed in order of decreasing inhibition of fr. wt and incremental changes calculated for peroxidase (Table 2).

Expressed on a fr. wt basis, salinity-induced changes in peroxidase vary among the cultivars of *Cucumis* as they do in *Brassica* but the variations are of lesser magnitude (Table 2). When calculated on the basis of portein content, however, peroxidase activity is reduced at least 18% in all but 2 out of 21 cases and in 17 of these cultivars, by more than 50%. This apparently selective reduction in peroxidase has also been observed in papaya and maize; in both instances a small elevation in enzyme activity based on fr. wt is contrasted with a modest to large reduction in the enzyme's specific activity. Thus, of 23 cultivars representing six species (three *Brassica* sp. are included), $\Delta\% > -50\%$ was found in 78% and some significant degree of reduction in 91%.

In this study plants were cultured at specific seawater dilutions quite near their upper tolerance limits for growth, and a change in the specific activity of a readily assayed enzyme seems to offer promise as a salt stress indicator where direct activity per unit fr. wt does not. The physiological mechanisms involved cannot be seriously considered without at least a comparative examination of heme-enzymes other than peroxidase. It is reasonable to assume, however, that although peroxidase and total protein may both be subject to salinity-induced increases in many cases, the point of optimum salt concentration lies at a lower value for enzyme syntheses than for proteins in general.

Table 1. Changes in fr. wt, protein content and peroxidase activity in cucumber seedlings cultivated 10 days in fresh water or one-third strength seawater

Cultivar	Fr. wt (mg/seedling)	Lowry protein (mg/g fr. wt)	Peroxidase Fr. wt (μg-eq/g)	Peroxidase Protein (μg-eq/mg)
1. Early Sure Crop Hybrid				
a fresh	188 ± 26	0.43	0.038	88.4
b sea	60 ± 16	1.42	0.048	33.8
ratio b/a	0.32	3.30	1.27	0.35
2. Lemon				
a fresh	225 ± 24	0.19	0.048	252.6
b sea	75 ± 18	0.57	0.052	91.2
ratio b/a	0.33	3.00	1.08	0.36
3. Straight Eight				
a fresh	190 ± 21	0.14	0.055	392.8
b sea	74 ± 16	0.98	0.055	56.1
ratio b/a	0.39	7.00	1.00	0.14
4. Pick of the Pickle				
a fresh	247 ± 31	0.66	0.056	84.8
b sea	107 ± 13	1.08	0.072	80.6
ratio b/a	0.47	1.64	1.55	0.14
5. Delila				
a fresh	226 ± 27	0.24	0.050	208.3
b sea	107 ± 13	1.03	0.072	70.0
ratio b/a	0.47	4.29	1.44	0.34
6. Lehua No. 1				
a fresh	244 ± 31	0.25	0.067	268.0
b sea	124 ± 16	0.94	0.063	67.0
ratio b/a	0.51	3.76	0.94	0.25
7. Chicago Pickling				
a fresh	238 ± 20	0.24	0.062	258.3
b sea	132 ± 14	0.76	0.055	72.4
ratio b/a	0.56	3.17	0.85	0.28
8. Early Russian				
a fresh	244 ± 26	0.51	0.062	252.6
b sea	142 ± 16	0.85	0.073	85.9
ratio b/a	0.58	4.47	1.53	0.34
9. Femqueen				
a fresh	224 ± 26	0.51	0.062	121.6
b sea	137 ± 15	0.90	0.091	100.9
ratio b/a	0.61	1.76	1.50	0.82
10. White Wonder				
a fresh	204 ± 21	0.55	0.068	122.6
b sea	129 ± 14	1.03	0.058	56.0
ratio b/a	0.63	1.87	0.89	0.46

EXPERIMENTAL

Fr. wt, protein and peroxidase (EC 1.11.1.7) were determined in seedlings of *Cucumis sativus* L. after 10 days culture at 24° under daylight fluorescent illumination at 100 μE/cm/sec. The same seedling homogenates were used for Lowry protein (BSA standard) and photometric peroxidase assay (guaiacol– H_2O_2 at 470 nm) as previously described [1]. Fr. wt data (mean ± s.d.) are based on 40–60 seedlings. Protein values are based on triplicates generally agreeing to *ca* ±10%; peroxidase values are based on triplicates generally agreeing to *ca* ±15%. For compartive purposes, previously published *Brassica* data as noted above, and results with *Zea mays* cv Hawaiian sugar [13] have been included together with recent results from experiments now in progress using *Carica papaya* cv Solo.

Brassica experiments were essentially like those with *Cucumis* but only 7 days in duration, as previously described. In contrast, glasshouse cv Solo papaya seedlings grown in Hoagland's nutrient for 8–12 weeks were transferred to saline nutrient media for 2 weeks followed by measurement and analysis. Each value represents the mean of nine plants (triplicate pots). Papaya was grown under natural diurnal light conditions with maximum sunlight intensities of *ca* 1000 μE/cm²/sec over a temperature range of 22–27° (August–October, 1980). *Zea* was fieldgrown under March–May conditions in 1978 as previously reported [13].

Table 2. Effect of cultivation in seawater dilutions on inhibition of fresh weight and differential values for peroxidase

Cultivar	Fr. wt inhibition %	Peroxidase* Fr. wt basis Δ%	Peroxidase* Protein basis Δ%
Brassica			
De Rapa Broccoli	91	−25	−85
Ferry's Round Dutch Cabbage	90	−31	−61
Red Dutch Cabbage	87	−15	−74
Neptune Broccoli	85	−10	−53
Pak Choi	78	56	−55
Savoy Chieftan	74	158	−18
Copenhagen Market Cabbage	73	−37	−77
Michihli	71	−55	−81
Danish Ball Head	68	−71	−92
Wong Bok	54	141	−4
Early Jersey Wakefield	46	492	−61
Cucumis			
Early Sure Crop Hybrid	68	27	−65
Lemon	67	8	−64
Straight Eight	61	0	−86
Pick of the Pickle	55	55	−5
Delila	53	44	−66
Lehua No. 1	49	−6	−75
Chicago Pickling	44	−11	−72
Early Russian	42	53	−66
Femqueen	39	50	−18
White Wonder	37	−15	−54
Carica papaya			
Solo	48	+19	−53
Zea mays			
Hawaiian sugar	63	+15	−20

Calculations of inhibition and differential values based on *Brassica* data in Stevens *et al.* [1] and on *Cucumis* data in Table 1.

*$\Delta\% = 100\left(\frac{\text{sea}}{\text{fresh}}\right) - 100.$

Protein was determined on seedling homogenates using the Lowry method (BSA as standard) [14] and peroxidase (EC 1.11.1.7) 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, 3000g/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 [15], and over the pH range 4.5–6.6.

Acknowledgements—This work was supported by grants from the State of Hawaii Marine Affairs Coordinator (Technical Order No. 187) and U.S. Department of Agriculture, Science and Education Administration, Section 406 (Project No. HAW00693-S).

REFERENCES

1. Stevens, H., Calvan, M., Lee, K., Siegel, B. and Siegel, S. (1978) *Phytochemistry* **17**, 1521.
2. Levitt, J. (1972) *Responses to Plants to Environmental Stress*. Academic Press, New York.
3. Siegel, S. (1968) *Adv. Space Sci. Technol.* **9**, 1.
4. Gerloff, E., Stahmann, M. and Smith, D. (1967) *Plant Physiol.* **42**, 1280.
5. Doyle, J. and Clinch, P. (1927) *Proc. R. Ir. Acad. Sect. B* **37**, 373.
6. Highkin, H. (1967) *Plant Physiol. Suppl.* **42**, 5.
7. De Jong, D., Olson, A., Hawker, K. and Jensen, E. (1968) *Plant Physiol.* **43**, 841.
8. Vieria-de-Silva, J. (1968) *C. R. Acad. Sci. Ser. D* **67**, 729.

9. Stutte, C. and Todd, G. (1967) *Phyton* **24**, 67.
10. Siegel, S., Giumarro, C. and Daly, O. (1966) *Nature (London)* **209**, 1330.
11. Rakova, N., Klyshev, L. and Strogonov, B. (1969) *Fisiol. Rast.* **16**, 22.
12. Strogonov, B. (1964) *Physiological Basis of Salt Tolerance in Plants.* Academic Science, U.S.S.R. (Davey, New York).
13. Siegel, S., Siegel, B., Massey, J., Lahne, P. and Chen, J. (1980) *Physiol. Plant.* **50**, 71.
14. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265.
15. Siegel, S. M., Speitel, T. and Stoecker, R. (1969) *Cryobiology* **6**, 160.