Phytochemistry, Vol. 26, No. 2, pp. 367–369, 1987.
Printed in Great Britain.

0031-9422/87 $3.00 + 0.00

GLYCINE–BETAINE ACCUMULATION INFLUENCES SUSCEPTIBILITY OF WATER-STRESSED BARLEY TO THE APHID *SCHIZAPHIS GRAMINUM*

GUSTAVO E. ZÚÑIGA and LUIS J. CORCUERA*

Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile

(*Revised received* 6 *June* 1986)

Key Word Index—*Hordeum*; Gramineae; barley; *Schizaphis graminum*; greenbug; aphids; insect resistance; water stress; betaine; proline.

Abstract—Water deficit increased susceptibility of barley to the aphid *Schizaphis graminum*. Proline and glycine–betaine accumulated in the stressed plants. These compounds were incorporated into artificial diets to test their effects on aphids. Survival of *S. graminum* was not affected by proline and glycine–betaine. In addition, glycine–betaine increased reproduction of the greenbug at concentrations similar to those found in stressed barley plants. When glycine–betaine was added to detached shoots of barley, population growth rate of *S. graminum* increased in that plant material kept in the betaine solutions. It is suggested that glycine–betaine accumulation may be responsible for the increased susceptibility of water-stressed barley to the greenbug.

INTRODUCTION

Barley leaves accumulate proline and glycine–betaine under water stress [1]. The grain yields of barley cultivars that accumulate proline as a response to water stress are not decreased over a wide range of environments [2]. However, proline accumulation does not serve as an index of drought resistance [3]. Although a precise role has yet to be assigned to glycine–betaine, this compound may function as an organic cytoplasmic osmoticum [4]. The finding that glycine–betaine is located in the cytoplasm supports this hypothesis [5]. Moreover, proline and glycine–betaine protect several enzymes against *in vitro* heat inactivation [6]. It has also been reported that proline concentration increases in virus-infested leaves [7]. Haglund [8] suggested that plants with a higher content of proline are more susceptible to insect predators but it was later shown that proline content does not influence susceptibility of barley [9]. Betaine and choline concentration increased following rust infection in susceptible but not in resistant wheat varieties [10]. However, a direct correlation between the susceptibility and the concentration of these compounds has not been demonstrated. We report here that accumulation of glycine–betaine in water-stressed barley may influence the susceptibility of seedlings to the aphid *Schizaphis graminum* (Rondani).

RESULTS AND DISCUSSION

Water stress and susceptibility of barley

Barley seedlings subjected to water stress were infested with adult apterous aphids. Aphid population growth rate increased in the stressed plants (Fig. 1a). These plants also accumulated glycine–betaine and proline (Fig. 1b). To test if these compounds were responsible for the increased susceptibility, detached shoots and leaves were put in solutions containing proline, choline and glycine–betaine. After two days each group was infested with adult aphids and the population growth rate was determined six days later (Fig. 2). Population growth rate increased in plants kept in the glycine-betaine solutions. Proline and choline produce a small decrease in the population growth rate.

Aphid feeding experiments

Proline, choline and glycine–betaine were incorporated into artificial diets to test their effects on the greenbug. Choline decreased survival of aphids, while proline and glycine–betaine did not (Table 1). Moreover, glycine–betaine increased reproduction of the greenbug at concentrations similar to those found in plants (Table 1 and Fig. 3).

Our results have shown correlations between natural accumulation of glycine–betaine and plant susceptibility. Also, a similar correlation was found when glycine–betaine concentrations of the leaves were artificially increased by putting the plants in glycine–betaine solutions. Moreover this compound increased the reproduction of aphids reared with artificial diets. Thus, it is suggested that glycine–betaine accumulation may be a factor responsible for increasing the susceptibility of water stressed barley to the aphid *S. graminum*. Whether other compounds involved in water stress responses also increase susceptibility to aphids remains to be determined. Since proline and choline did not show beneficial effects on aphids reared on artificial diets, it is possible that cultivars which preferentially accumulate these compounds are more resistant to aphids than those that accumulate glycine–betaine.

*To whom correspondence should be addressed.

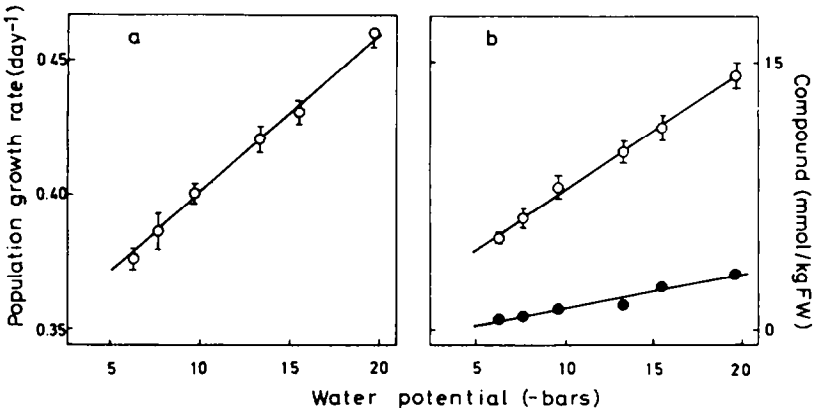

Fig. 1. Effect of water stress on the susceptibility of barley plants and proline and glycine–betaine content. Barley seeds cv F. Union, were grown in soil a 16 hr photoperiod in a growth chamber at 25 ± 2°. Four-day-old seedlings were kept without irrigation for 48 hr. Five groups of plants were then irrigated daily with different amounts of water for six days. Leaf water potentials were measured in ten-day-old seedlings using a pressure chamber. Replicate groups were infested with adult apterous aphids. Insect population growth rate was determined six days after (Fig. 1a). Each value is the mean of three samples of ten plants each. Vertical bars are 1 s.e. Population growth rate $= \ln(Nf/Ni)/\Delta t$. Proline (●) and betaine (○) (Fig. 1b) were quantified as described in the experimental section.

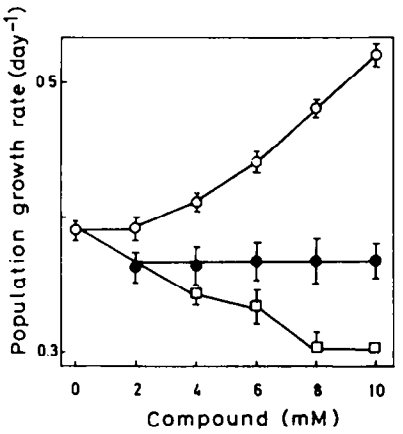

Fig. 2. Effect of proline, choline and glycine–betaine incorporated by detached shoots of barley on the susceptibility to aphids. Detached shoots of eight-day-old barley seedlings cv F. Union, were put in solutions containing proline, choline and glycine–betaine. After two days each group was infested with two adult apterous aphids. Population growth rate was determined six days after, as in Fig. 1. Each value is the mean of three samples of five plants each. Vertical bars are 1 s.e. Solutions contained 10^{-6} M kinetin to delay senescence of leaves. At this concentration kinetin did not have detectable effects on the greenbug. Glycine–betaine (○); proline (●); choline (□).

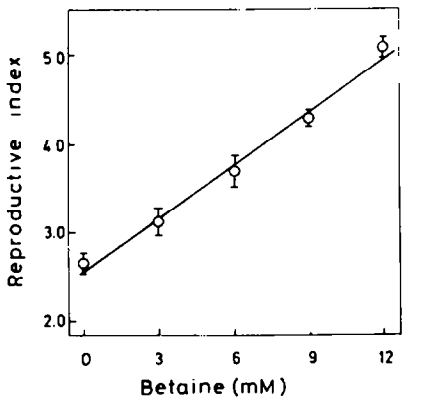

Fig. 3. Effect of glycine–betaine on reproduction of *S. graminum* reared on artificial diets. Adult aphids were fed with artificial diets with glycine–betaine added. The reproductive index was measured after 72 hr. Each value is the mean of three samples of ten aphids. Vertical bars are 1 s.e. The reproductive index was the amount of nymphs born divided by the average number of adults. All values were significantly different at $P < 0.05$ by t test.

EXPERIMENTAL

Analyses of compounds. Proline was quantified by the method of Bates *et al.* [11]. Plant material (0.5 g) was homogenized in 10 ml of 3% aqueous sulphosalicyclic acid and then filtered through Whatman No. 1 filter paper. Two ml of this filtrate were incubated with 2 ml acid-ninhydrin and 2 ml HOAc at 100° for 30 min and the reaction terminated in an ice bath. The reaction mixture was extracted with 4 ml toluene and the absorbance was measured at 520 nm using toluene for a blank ($\varepsilon_{520} = 11\,511$).

Glycine–betaine was quantified by a method previously described [12]. Dried plant material (0.5 g), was shaken with 20 ml of deionized H_2O for 24 hr at 25°. The extracts were diluted 1:1 with 2 N H_2SO_4. Aliquots of 0.5 ml were cooled in ice water for 1 hr and then 0.2 ml of cold KI–I_2 reagent was added. The tubes were stored at 0–4° for 16 hr and then centrifuged at 5,000 *g* for 30 min at 0°. The supernant was discarded. The periodide crystals were dissolved in 5 ml 1,2-dichloroethane. After 2.5 hr the absorbance was measured at 365 nm ($\varepsilon_{365} = 2244$).

Water stress treatment. Five groups of four-day-old seedlings were watered daily with different amounts of water for six days. Water potential of leaves was measured at this stage [13]. Then, each group of plants was infested with adult apterous aphids of *S. graminum*. The number of aphids on the leaves was measured six

Table 1. Effects of proline, choline and glycine-betaine on *Schizaphis graminum* feeding on artificial diets

Compound	Survival (%)	Amount of diet ingested (mg/10 aphids)	Reproductive index (nymphs/adult)
None	93 ± 3	0.9	2.5 ± 0.3
Proline	96 ± 6	0.9	2.3 ± 0.1
Choline chloride	72 ± 5*	0.7*	2.2 ± 0.2
Glycine-betaine	98 ± 3	0.9	3.3 ± 0.2*

Biological assays were performed with holidic diets placed between two layers of parafilm M. The diet was as described in the Experimental with 6 mM of each compound added. Survival of aphids was measured after 48 hr of feeding. The amounts of diet ingested were measured by weighing the parafilm sachets after 7 hr. The reproductive index was measured after 72 hr. All assays were performed with adult apterous aphids. Values shown are the mean of three samples of ten aphids each ± 1 s.e. Reproductive index is the amount of nymphs born divided by the average number of adults. (*) = significant differences from control values at $P < 0.05$ by t test.

days after and population growth rate was calculated (population growth rate = $\ln(Nf/Ni)/\Delta t$).

Feeding assays. Assays were performed with diets placed between two layers of Parafilm M[14]. The diet was a solution containing 35% sucrose, amino acids and mineral salts adjusted to pH 6.0. Three samples of ten aphids each were used for each treatment.

Acknowledgements—This work was supported by grants from the Agency for International Development, Universidad de Chile (N-1654) and Fondo Nacional de Ciencias. We thank Ing. Agr. Edmundo Beratto and Compañia Cervecerías Unidas for providing the seed used.

REFERENCES

1. Hanson, A. D. (1980) *Hort. Sci.* **15**, 623.
2. Singh, T. N., Aspinall, D. and Paleg, L. G. (1972) *Nature, New Biol.* **236**, 188.
3. Hanson, A. D., Nelsen, Ch. E. and Everson, E. H. (1977) *Crop Sci.* **17**, 720.
4. Wyn Jones, R. G., and Storey, R. (1981) *The Physiology and Biochemistry of Drought Resistance in Plants* (Paleg L. G. and Aspinall, D., eds.). Academic Press, Sidney.
5. Hall, J. L., Harvey, D. M. R. and Flowers, T. J. (1978) *Planta* **140**, 59.
6. Paleg, L. G., Douglas, T. J., van Daal, A. and Keech, D. B. (1981) *Aust. J. Plant Physiol.* **8**, 107.
7. Stewart, G. R. and Larher, I. (1980) *The Biochemistry of Plants*, Vol. 5 (Miflin, B. J., ed.). Academic Press, New York.
8. Haglund, B. M. (1980) *Nature* **288**, 697.
9. Bright, S. W. J., Lea, P. J., Kueh, J. S. H., Woodcock, C., Holloman, D. W. and Scott, G. C. (1982) *Nature* **295**, 592.
10. Strange, R. N., Mayer, J. R. and Smith, H. (1974) *Physiol. Plant Path.* **4**, 277.
11. Bates, L. S., Waldren, R. P. and Teare, I. D. (1973) *Pl. Soil* **39**, 205.
12. Grieve, C. M. and Grattan, S. R. (1983) *Pl. Soil* **70**, 303.
13. Scholander, P. F., Hammel, H. T., Hemmingsen, E. S. and Brandstreet, E. D. (1964). *Proc. Nat. Acad. Sci., U.S.A.* **52**, 119.
14. Argandoña, V. H., Peña, G. F., Niemeyer, H. M. and Corcuera, L. J. (1982) *Phytochemistry* **21**, 1573.