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GLYCINE-BETAINE IN WILTED BARLEY REDUCES THE EFFECTS OF GRAMINE ON APHIDS

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Key Word Index—*Hordeum*; Gramineae; barley; water stress; aphids; betaine; proline; gramine; *Schizaphis graminum*; greenbug; *Rhopalosiphum padi*.

Abstract—The gramine content in different barley cultivars was not changed under water stress. Proline and glycine-betaine increased as plant water potential decreased. The population growth rate of the aphids *Schizaphis graminum* and *Rhopalosiphum padi* were increased in plants with higher glycine-betaine/gramine ratio. When the aphids were fed with artificial diets with different ratios of glycine-betaine/gramine, the survival of aphids increased in proportion to the glycine-betaine content in the diets. Proline had no effect on the survival of aphids. The LD_{50} of gramine was 0.8 and 4.1 mM when 0 and 12 mM glycine-betaine were added to the diets. These results suggest that glycine-betaine accumulation due to water stress may reduce the harmful effects of gramine on the aphids *S. graminum* and *R. padi*.

INTRODUCTION

The identification of factors that regulate plant–insect interactions may be important for breeding pest-resistant cultivars. For example, gramine and related indole alkaloids are responsible for toxicity of *Phalaris sp* to ruminants [1, 2], and gramine is a factor in the resistance of several barley cultivars to the aphids *Schizaphis graminum* and *Rhopalosiphum padi* [3, 4]. Gramine concentration in barley shoots declines with age in well-watered plants grown at moderate temperatures [5]. On the other hand, the gramine content in barley plants under water stress does not change [6]. However, barley leaves may accumulate proline and glycine-betaine in response to water stress [7]. We have previously reported that accumulation of glycine-betaine in water stressed barley may increase its susceptibility to the aphid *S. graminum* [8]. In this work we report that in barley cultivars that contain gramine, the accumulation of glycine-betaine in response to water stress may reduce the harmful effects of gramine, increasing the aphid population on the plants.

RESULTS

Proline, glycine-betaine and gramine in plants under water stress

Measurements of leaf water potentials showed that gramine content remained nearly unchanged in water stressed and unstressed leaves (Table 1). However, the proline and glycine-betaine content were higher in stressed leaves of barley. Accumulation of proline, glycine-betaine and gramine in leaves depended also on the cultivars used.

Effects of water stress on barley susceptibility

We have previously shown correlations between natural accumulation of glycine-betaine and plant susceptibility to *S. graminum* in a gramine-free barley cultivar [8]. To determine the effects on aphids produced by gramine and glycine-betaine in stressed plants, seedlings of different barley cultivars were infested with adult apterous nymphs of *S. graminum* and *R. padi*. The population growth rate of aphids on the more stressed plants was lower in those cultivars with higher gramine content (Fig. 1). However, in stressed barley seedlings, it was observed that the population growth rate of both species of aphids increased with respect to the normal seedlings. Since proline did not show significant effects on *S. graminum* [8] it is likely that the observed effects are due to glycine-betaine accumulation. Soluble sugars in both treatments did not change significantly (not shown).

Effects of gramine and glycine-betaine on aphids

Gramine decreased survival of *S. graminum* and *R. padi* with a similar LD_{50} (0.8 mM) [3, 4]. When the aphids were fed with diets with a constant gramine concentration and different glycine-betaine concentrations, the survival of both species of aphids increased (Fig. 2). These results suggested that glycine-betaine reduced the harmful effects of gramine on the insects. Proline was not effective in reducing gramine effects on aphids (not shown). The LD_{50} of gramine for *S. graminum* fed with artificial diets increased in proportion to the glycine-betaine content in the diets (Fig. 3). This effect was observed on aphids with gramine and glycine-betaine at concentrations normally found in plants.

DISCUSSION

The results shown here and those previously reported [8], suggested that glycine-betaine accumulation may

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Table 1. Effects of water stress on gramine, proline and glycine-betaine content in barley seedlings

Cultivar	Water potential (− bars)	Compound (mmol/kg dry wt) Gramine	Proline	Glycine-betaine
F. Union	5.3	N.D.	8 ± 1	18 ± 2
	12.0	N.D.	30 ± 3	90 ± 8
Cruzat	5.6	20 ± 2	20 ± 2	54 ± 4
	12.0	19 ± 2	31 ± 3	94 ± 8
MCU-34	5.6	37 ± 3	7 ± 1	31 ± 2
	12.0	30 ± 3	37 ± 3	50 ± 4

Barley seedlings, were grown in soil under continuous light in a growth at 25 ± 2∂. Two groups of six-day-old seedlings were irrigated daily with different amounts of water for six days. Leaf water potentials were measured in 12-day-old plants using a pressure chamber. Each value is the mean of two samples ± 1 s.e. The compounds were analysed as described in Experimental.

increase the susceptibility of barley seedlings to aphids. In general, it has been assumed that glycine-betaine is a solute involved in osmotic adjustments [9]. This compound has been shown to be a major osmoticum in halophilic organisms [10]. Moreover, glycine-betaine prevents the inhibition of several enzyme activities by high concentration of salts [11].

Barley may be an important crop in some dry areas [12]. In periods of water deficit barley accumulates proline and glycine-betaine [7]. It has been proposed that stress-induced glycine-betaine accumulation is an adaptative metabolic response [13, 14], and that its accumulation could be a valuable metabolic criterion in breeding for drought resistance [15]. However, in spite of the possible beneficial effects of glycine-betaine on plant metabolism under water stress, the results shown here indicated that a higher accumulation of this compound may also increase the susceptibility of barley to aphids in cultivars with or without gramine. Proline, another metabolite involved in responses of plants to stress, did not change the effects of gramine. Since gramine content does not change with water stress and high glycine-betaine content increases the susceptibility of plants to aphids, it is possible that plants that accumulate none or small amounts of glycine-betaine are more resistant to aphids that those plants without or reduced concentration of gramine and high glycine-betaine content.

The mechanism involved in insect protection against gramine by glycine betaine is unknown. However, gramine has inhibitory effects on mammalian mitochondria and submitochondrial particles [16]. Respiration of the bacterium *Pseudomonas syringae* was also inhibited by gramine. Glycine-betaine totally reversed this inhibition [17], and, it is possible that glycine-betaine may somehow affect cellular respiration of aphids.

EXPERIMENTAL

Plant material and infestation with aphids. Three barley cultivars were analysed (F. Union, Cruzat and MCU-34). Seeds were sown in pots filled with soil. Plants were grown under continuous light at 25° in a growth chamber. Six-day-old plants of each cultivar were watered daily with different amount of H_2O for 6

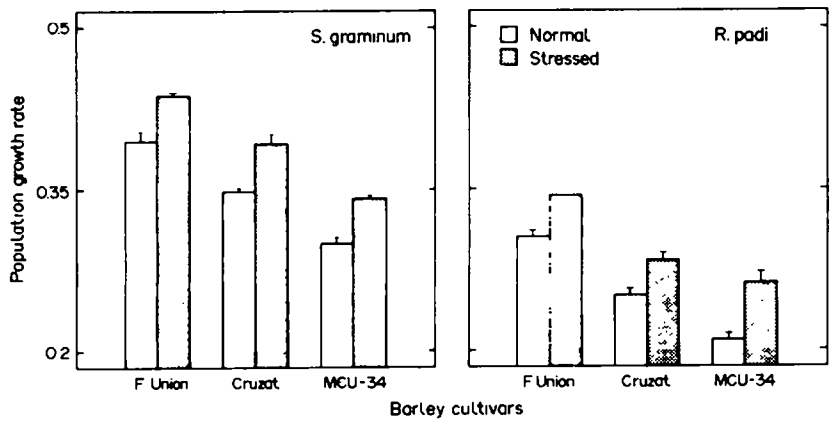

Fig. 1. Water stress and susceptibility of barley to aphids. Barley seed cv F. Union, Cruzat and MCU-34 were grown in soil under continuous light in a growth chamber at 25 + 2°. Six-day-old seedlings were subjected to water stress as described in Experimental. Each of these plants were infested with two nonalate adults. Aphids were counted after six days. Each value is the mean of three samples of ten plants each. Vertical bars are 1 s.e. Population growth rate $= \ln (N_f/N_i)/\Delta t$ (per day).

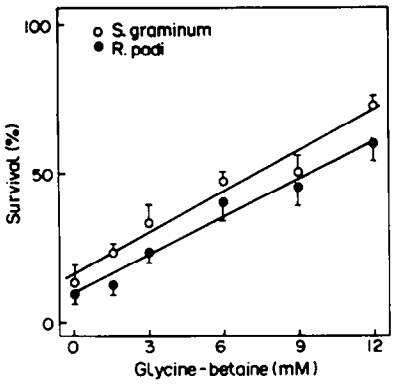

Fig. 2. Effects of glycine-betaine of survival of aphids fed in diets with gramine. Aphids were fed with artificial diets with 3 mM gramine and glycine-betaine at various concentration. Survival was measured after 48 hr. Each value is the mean of three samples of ten aphids. Vertical bars are 1 s.e.

days. Plant water potentials were measured at this state of development using the methodology described [8]. Each group of plants was infested with adult apterous aphids of *S. graminum* and *R. padi*. The increase in the insect population was determined after 6 days and population growth rate was calculated (Population growth rate = $1n(N_f/N_i)/\Delta t$).

Analyses of compounds. For proline analyses, leaf samples were homogenized in 10 ml of 3% aq. sulphosalicyclic acid and quantified by the method described in ren. [18]. Glycine-betaine was quantified by a method described in ref. [19]. Dried plant material (0.5 g), was shaken with 20 ml of deionized H_2O for 24 hr at 25° and then filtered. The extract was diluted 1:1 with 2 N H_2SO_4. Aliquots of 0.5 ml were cooled in ice H_2O for 1 hr and then 0.2 ml of cold KI–I_2 was added. The tubes were stored at 4° over night and then centrifuged at 10000 *g* for 15 min. at 0°. The supernant was discarded and the ppt. was dissolved in 5 ml 1,2 dichloroethane. After 2.5 hr the *A* was measured at 365 nm.

Gramine measurements were performed as described previously [4]. Sugars were extracted in 10 ml of 80% (v/v) EtOH at 25° with constant shaking. Total soluble sugars were quantified as described in ref. [20]. 0.1 ml of the alcoholic extract was reacted with 3 ml of freshly prepared anthrone at 100° for 10 min. The reaction was stopped in ice and the *A* of the samples was measured at 625 nm.

Feeding assays. Assays were performed with diets placed between 2 layers of Parafilm M [21]. The diet was a soln containing 35% sucrose, aminoacids and mineral salts adjusted to pH 6. Three samples of 10 aphids were used for each treatment.

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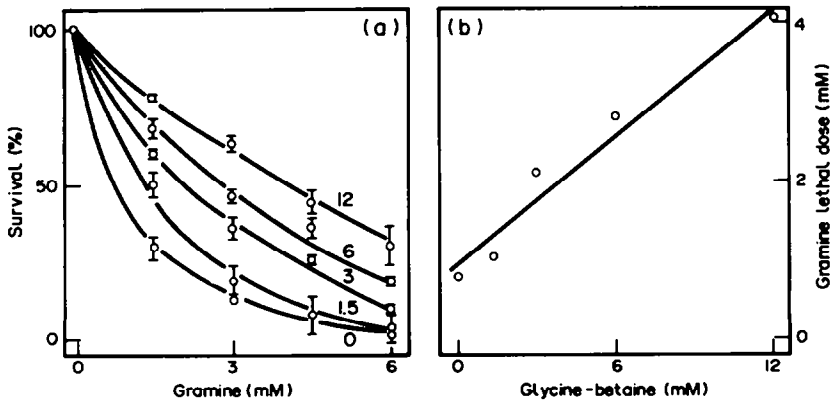

Fig. 3. Survival of S. graminum in diets with gramine and glycine-betaine Adult aphids were fed with artificial diets containing various amount of gramine and glycine-betaine. Survival was measured after 48 hr (3-A). The numbers over the curves indicate glycine-betaine concentration (mM). The lethal dose of gramine as affected by glycine-betaine was then calculated (3-B).

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