Betaines in the Plant Kingdom and Their Use in Ameliorating Stress Conditions in Plants

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Abstract

Betaines have a widespread distribution in the plant and animal kingdoms. The most studied role of these compounds is that of compatible osmolytes, facilitating adaptation to saline and dry environments. Betaines can be accumulated to levels greater than 5 µmol g^{-1} dry weight, at which concentration they are likely to function as compatible osmolytes. However, many species contain these compounds in very much lower concentrations and their role in these cases has not been fully elucidated.

Recent studies on the application of betaines in low amounts to plants has resulted in significant enhancement of the ability of the treated plants to resist stress conditions. For example, application of glycinebetaine, γ-aminobutyric acid betaine and δ-aminovaleric acid betaine to dwarf French bean, tomato, wheat and barley plants led to significantly higher leaf chlorophyll levels in the treated plants in comparison to the controls. Application of the same betaines to tomato plants also resulted in significantly reduced invasion of the roots of the plants by second stage juveniles of the root knot nematodes, *Meloidogyne javanica* and *M. incognita*. Egg recovery from the roots of the treated plants was also significantly reduced. Application of betaines to plants in low concentrations has also led to significantly lower levels of fungal attack.

Application of [^{14}C]-glycinebetaine to leaves of turnip rape plants resulted in the labelled compound being detected in the roots within two hours of application. Within one day after application, the labelled glycinebetaine had been translocated to all plant parts.

Treatment of wheat plants with glycinebetaine led to a significantly increased tolerance to freezing stress. The glycinebetaine treatment resulted in the induction of a subset of low temperature responsive genes that are also induced by salinity and drought stresses.

INTRODUCTION

Betaines are derivatives of amino acids containing a fully methylated quaternary nitrogen moiety. Compounds of this class have been found in many animals, plants, algae, fungi and bacteria (Blunden & Gordon, 1986). The most investigated role of betaines is that of compatible osmolytes aiding adaptation to saline and dry conditions. For this use, however, the concentrations of the compounds must be high, for example, as in the Chenopodiaceae (Adrian-Romero et al., 1998) and Amaranthaceae (Blunden et al., 1999), but in many betaine-containing organisms, the levels of these compounds are low, as in the Polygonaceae (Adrian-Romero et al., 1998) and Bromeliaceae (Adrian-Romero et al., 2001). In recent years it has been demonstrated that betaines have a role in aiding plants to resist stress conditions, such as attack by pathogens, frost and heat damage, and growing in saline soils. Evidence for this is presented in this communication.

BETAINES IN HIGHER PLANTS

The most commonly reported betaine is glycinebetaine (1), which has been found in a wide range of plant families, including the Chenopodiaceae, species of which can

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accumulate large concentrations of this compound; for example over 3 % of the dry weight of *Atriplex littoralis* (Adrian-Romero et al., 1998). Many species, however, contain only trace amounts of glycinebetaine, for example *Dyckia ferox* (0.001 %, dry weight) (Adrian-Romero et al., 2001).

B-Alaninebetaine (2) has a much more restricted distribution, but is a feature of the Plumbaginaceae (Rhodes et al, 1993). Laminine (3), a derivative of lysine, has been found in the free state in some plants and also as a component of proteins (Tyihák, 1969). It is an unusual compound, being both a quaternary ammonium derivative and an α-amino acid capable of forming peptide bonds. Phenylalaninebetaine (4) has been isolated from *Antiaris africana* (Moraceae) by Okogun et al. (1976).

Prolinebetaine (5) occurs widely in nature and has been found in many higher plants, for example the Labiatae/Lamiaceae (Blunden et al., 1996). Both its *trans*-4-hydroxy- (6) and *trans*-3-hydroxy- (7) derivatives have also been reported, the former in, for example, the Labiatae/Lamiaceae (Blunden et al., 1996) and the latter in, for example, the Capparaceae (Mc Lean et al., 1996).

Pipecolic acid betaine (8) and *trans*-4-hydroxypipecolic acid betaine (9) have both been isolated from *Lamium* species and baikianbetaine (10) has also been reported.

The picolinic acid betaines, trigonelline (11) and homarine (12) have been recorded for higher plants. The former has a widespread distribution, but the latter appears to be much more restricted.

From Gymnosperm species, leucinebetaine (13) has been isolated from *Cycas circinalis* seeds (Li et al., 1996) and tyrosinebetaine (14) from *Ephedra* species (Tamada et al., 1978).

BETAINES IN FUNGI AND LICHENS

Glycinebetaine, trigonelline, homarine and tyrosinebetaine, all recorded above, have been reported for fungi. Glycinebetaine was found to accumulate to high levels and a content as high as 7.8 %, dry weight, was recorded for *Agaricus arvensis* (Andreeva, 1971). Histidinebetaine (15) and thiohistidinebetaine (16) have been found in many fungal species (for example, List 1958), and clithioneine (17) was isolated from *Clitocybe acromelaga* (Konno et al., 1981). Γ-aminobutyric acid betaine (18) has been recorded for *Polyporus sulphureus* by List (1958). Carnitine (19) has recently been found in several species of fungi, including *Coriolus versicolor*, *Coprinus micaceus* and *Amanita muscaria* (Tyihák et al., unpublished).

From the lichen, *Lobaria laetevirens*, the methyl ester of dihydroxyphenyl-alaninebetaine (20) was isolated and characterized, along with dihydroxyphenyl-alaninebetaine (DOPA betaine) and tyrosinebetaine (Bernard et al., 1980).

BETAINES IN MARINE ALGAE

Betaines and their sulphonio analogues reported for marine algae were reviewed by Blunden et al. (1986) and later by Blunden et al. (1992). Of the compounds mentioned above, glycinebetaine, β-alaninebetaine, γ-aminobutyric acid betaine, laminine, prolinebetaine, *trans*-4-hydroxyprolinebetaine and and homarine have all been isolated from marine algae. Other betaines have also been found. These include δ-aminovaleric acid betaine (21) and its methyl ester, α-alaninebetaine (22), N-acetyllaminine, 6-amino-6-carboxy-2-trimethylammoniohexanoate (23), β-prolinebetaine (24) and its *trans*-4-hydroxy derivative (25).

In addition to betaines, tertiary sulphonium compounds also occur, in particular in the Chlorophycota. The most widely distributed substance of this type is 3-dimethylsulphoniopropionate (26)

EFFECTS OF BETAINE APPLICATION TO PLANTS

Betaines and other related quaternary ammonium compounds can act as antistressors in both biotic and abiotic stress conditions. For example, glycinebetaine has been shown to enhance water utilization efficiency of winter wheat (Bergmann et al., 1984), to provide partial protection of some enzymes against salt inhibition (Pollard et al, 1979), and to have a role in frost resistance of plants (Bokarev et al, 1971). Several studies have also been undertaken on the role of betaines and other quaternary ammonium compounds in increasing the resistance of plants to attack by fungi. Tyihák et al. (1988) linked the resistance of tomato plants to *Fusarium oxysporum* to their content of quaternary ammonium compounds, in particular trigonelline, glycinebetaine and choline. Trigonelline has also been demonstrated to be a potential resistance inducer in plants against obligate biotrophic fungi (Kraska et al., 1992).

Experimental results that have advanced the knowledge of the effects produced when betaines are applied to plants have come from studies made using seaweed extracts and suspensions. The former products are prepared by extraction of the dried plant material with either water or aqueous alkali, whereas the suspensions are produced from fresh algae. *Ascophyllum nodosum* is the most widely used alga in Europe and North America, although small quantities of *Fucus* and *Laminaria* species are also utilised. In South Africa, *Ecklonia maxima* is the most common starting material and in Australia it is *Durvillaea potatorum*.

Many different effects have been reported as a result of the use of seaweed extracts, including increased crop yields, increased uptake of inorganic nutrients from the soil, reduced storage losses of fruit and increased resistance to stress conditions. The last effect is of particular interest and includes reduction in the incidence of fungal and insect attack and increased resistance to frost damage (Blunden et al., 1994). In 1984, Blunden et al. had suggested that betaines present in the extracts may be responsible for some of the reported effects. From the algae used to prepare seaweed extracts, γ-aminobutyric acid betaine, δ-aminovaleric acid betaine and laminine were isolated from *A. nodosum* and γ-aminobutyric acid, glycinebetaine and laminine from *Fucus* and *Laminaria* species (Blunden et al., 1986). Examination of commercial seaweed extracts has demonstrated that they contain the betaines present in the algae from which they have been prepared. Glycinebetaine is, however, a consistent constituent of the *A. nodosum* derived extracts as the result of the inevitable contamination of the starting material with other algae, such as *Fucus serratus.*

Enhanced Chlorophyll Levels. It had been observed that application of an *A. nodosum* based extract either to the soil or to the foliage of tomato plants produced leaves that, after 34 days, were visually more green than those of the control plants. The possible role of betaines in producing this result was considered and the effect on leaf chlorophyll content was investigated using a cucumber bioassay procedure devised for cytokinins (Fletcher,1982). The seaweed extract was shown to increase the chlorophyll levels of the cucumber cotyledons. The betaines in the seaweed extract, when tested separately, also produced significantly enhanced chlorophyll concentrations in the cotyledons and it was concluded that the effects of enhancing chlorophyll levels was due, at least in part, to the betaines it contains (Whapham et al., 1993). This study was extended by examining the effects on leaf chlorophyll of various species due to either foliar or soil application of either seaweed extract or a mixture of the betaines in the same concentrations as those present in the extract. Application of the seaweed extract resulted in higher concentrations of chlorophyll in the leaves of the treated plants (tomato, dwarf French Bean, wheat, barley, maize) in comparison to the controls. When the betaines were applied, very similar leaf chlorophyll levels were recorded for the seaweed extract and betaine treated plants. This suggests strongly that the enhanced chlorophyll content of plants treated with seaweed extract is dependent on the betaines it contains. Table 1. shows the leaf chlorophyll levels of dwarf French bean leaves after soil application of either seaweed extract or betaine solution. More detailed results can be found in Blunden et al. (1997).

Effects on Root Knot Nematodes. Soil application to the roots of tomato plants of an *A. nodosum* based extract resulted in a significant reduction in the number of second-stage juveniles of both *Meloidogyne javanica* and *M. incognita* invading the roots, compared to those of plants treated with water alone. Egg recovery from the seaweed extract treated plants was also significantly lower. The three major betaines found in the extract, when applied at concentrations equivalent to those present in the extract, also led to significant reductions in both the nematode invasion profile and egg recovery. This led to the conclusion that the betaines present in the seaweed extract play a major role in bringing about the observed effects. Detailed results can be found in Jenkins el al. (1998). Table 2. summarises some of the data.

Application of Glycinebetaine

Mäkelä et al. (1996) applied [^{14}C] glycinebetaine to the leaves of turnip rape plants and monitored them autoradiographically. They detected the compound in the roots within 2 hours of application and, one day after treatment, the labelled glycinebetaine had been translocated to all plant parts.

Agboma et al. (1997) treated drought-stressed tobacco plants with 0.1 and 0.3 M solutions of glycinebetaine and found that this led to significant increases in both fresh and dry weights of leaves. The glycinebetaine content of the plants remained high 16 days after application. Mäkelä et al. (1998) applied glycinebetaine to tomato plants grown either in saline soils or exposed to high temperature. This treatment led to significant increases in fruit yield. They also found that application of glycinebetaine to tomato plants grown under normal greenhouse conditions led to increased tomato yields.

Allard et al. (1998) reported that treatment of wheat plants with glycinebetaine resulted in large increases in the tolerance of the plants to freezing stress. A 4 day exposure to a 250 mM solution resulted in improvement in the freezing tolerance by more than 5^{o}C. Of particular interest was the observation that the treatment induced a subset of low temperature responsive genes that are also induced by salinity and drought stresses. The data suggest that glycinebetaine improved the freezing tolerance of the plants by eliciting some of the genetic and physiological responses associated with cold acclimation.

CONCLUDING REMARKS

It has been clearly demonstrated by many workers that application of betaines to plants leads to them having increased resistance to a number of stress conditions. However, the quantities applied in several of the experiments have been very low and would be less than the concentrations present in some of the treated plants. However, evidence has been produced that application of glycinebetaine to wheat plants led to the induction of a subset of low temperature responsive genes; these are also induced by salinity and drought stresses. It thus suggests that application of betaines stimulates the plant to produce its own defence mechanisms. This is consistent with the finding that compounds such as trigonelline are potential resistance inducers in plants against obligate biotrophic fungi. It may be that to stimulate the defence procedures the betaines have to be applied to the exterior of the plant. The plants' own betaines would come into contact with the surface of the plant should it be attacked by, for example root knot nematodes, or should the plant be subjected to frost damage and rupture of cells. Much work remains to be undertaken to take this subject further.

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Tables

Table 1. Leaf chlorophyll concentrations (mean SPAD units) of dwarf French bean leaves after soil application of either seaweed extract or betaine solution {*statistically significant difference (p=0.05) between control and test plants}

Time (days) after treatment		Control	Seaweed Extract	Betaine solution
0	Mean SPAD Units (MSU)	35.57	34.40	34.38
	Standard error (SE)	0.62	0.70	0.28
	Probability *p*		0.88	0.93
41	MSU	32.98	34.60	35.23
	SE	1.3	0.73	0.96
	P		0.16	0.10
49	MSU	30.07	32.75	34.00
	SE	1.2	1.2	0.96
	P		0.077	0.017*
57	MSU	27.03	29.20	29.49
	SE	0.56	0.49	0.67
	P		0.0084*	0.01*
63	MSU	24.92	27.70	27.30
	SE	0.61	1.00	1.00
	P		0.024*	0.04*
69	MSU	20.68	26.48	23.60
	SE	0.91	0.40	0.28
	P		0.0006*	0.014*

Table 2. Mean number (± standard error) of second stage juveniles (J2s) of *Meloidogyne incognita* and *M. javanica* per tomato plant 14 days post-inoculation (values for the mean number of eggs per plant 63 and 49 days post-inoculation, respectively, are given in brackets (*p*= probability)

Nematode species	Mean number of J2s and (eggs) per plant		
	Control	Seaweed extract	Betaine mixture
M. incognita	106.2 ±15.0 (11400 + 1800)	10.4 ± 6.4 $p < 0.001$ (660 ± 157) $p < 0.05$	23.4 ± 4.9 $p < 0.001$ (1460 ± 448) $p < 0.05$
M. javanica	89.2 ± 11.0 (18540 ± 1569)	9.6 ± 3.5 $p < 0.01$ (4600 ± 899) $p < 0.001$	68.0 ± 9.2 $p < 0.096$ (3260 ± 773) $p < 0.001$

Figures

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n = 2 = 2
n = 3 = 18
n = 4 = 21

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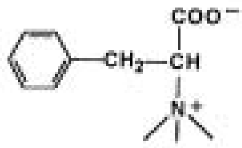

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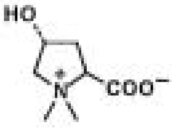

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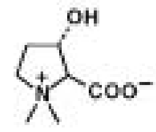

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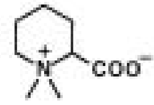

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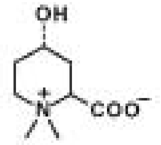

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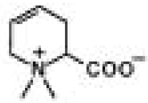

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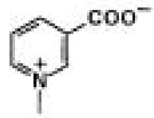

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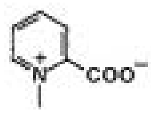

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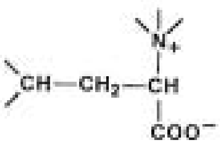

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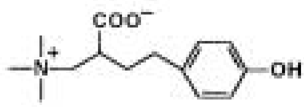

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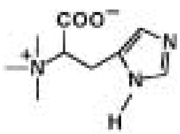

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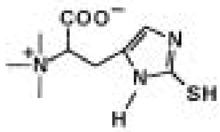

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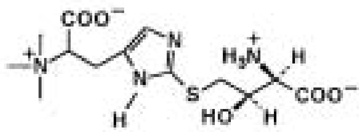

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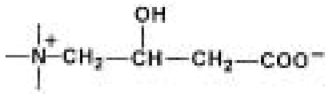

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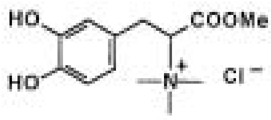

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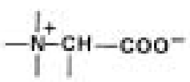

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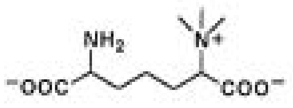

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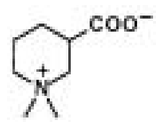

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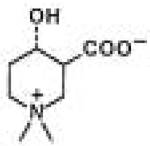

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