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GLYCINE BETAINE COUNTERACTS THE ANTAGONISTIC EFFECTS OF SALT STRESS ON PROTEIN METABOLISM

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ABSTRACT

Glycine betaine is a compatible organic metabolite which is synthesized and accumulated in pulses, cereals, grasses and many microbes in response to different types of stress. It has been shown to exhibit enhanced tolerance of plants to salt stress. In this investigation we examined the effects of salt stress and synthesis of glycine betaine on leaf protease activity in lucerne plants. Sodium chloride stress was observed to antagonise the stability of proteins by reducing protease activity and glycine betaine to reverse this antagonistic effect. This may be ascribed to the protection of machinery required for synthesis of proteins under salt stress. These findings suggest that glycine betaine counteracts the negative effects of salt stress probably with its accelerated.

KEYWORDS: Glycine betaine, Salt stress, Protein metabolism

Suboptimal environmental conditions constitute stress which affects many facets of plant metabolism adversely. There has been only a limited success in protecting the plants from soil toxicity by following the genetical, chemical or ecological approaches.

Glycine betaine is a compatible organic metabolite extremely soluble in water and of amphoteric nature. The molecular features of glycine betaine allow it to interact with both the hydrophilic and hydrophobic domains of macromolecules. This compound is found in a wide variety of higher plants, animals and both prokaryotic and eukaryotic microorganisms (Wyn Jones and Storey, 1978; Yancey et al., 1982, Hanson et al., 1985, Varshneyet al., 1988; Rathor, 2006).

Comprehensive studies, about the actions of glycine betaine, have shown that this compound induces the tolerance in plants to various environmental stresses such as excessive salt, cold, heat, drought and freezing (Sakamoto and Murata, 2001, Chen and Murata, 2002, Rathor, 2006, Varshney, 2006).

The present work, therefore, was conducted to evaluate the role of glycine betaine in protecting the proteins in lucerne plants through protease enzyme activity from being damaged by salt stress. On the basis of our results we discuss the response of lucerne plants to salt stress and glycine betaine.

MATERIALS AND METHODS

Purification of Protease

The lucerne plant leaves are procured from 70 days old plants and allowed to stand for two days in a humidity controlled dark growth chamber at 34^{0}C. The leaves were homogenized with 0.1M phosphate buffer (pH 7.0) containing 0.4M sucrose and 0.05M sodium ascorbate in a waring blender. Filtered homogenate was (I in Table 1) was squeezed through a gauze and centrifuged at 2000 rpm for 20 mins. The supernatant (II in Table 1) was adjusted to pH 5.0 by the addition of 1N acetic acid and allowed to stand overnight. The precipitate was removed by centrifugation and the supernatant (III in Table 1) was subjected to ammonium sulphate fractionation between 0.25 and 0.9 saturation. The ammonium sulphate precipitate was suspended in 0.0025M Mcilvaine buffer (pH 5.5) containing 2.5×10^{-3} M sodium ascorbate and dialyzed against the same buffer at 4^{0}C for 20 hrs. The dialysate (IV in Table 1) was centrifuged and the supernatant was fractionated by the addition of ethanol at -5^{0}C. A fraction precipitating between 20 and 60% ethanol was collected and dialyzed in the same way. Calcium phosphate gel (approximately 7 times w/w of protein content) prepared by the method of Keilin and Hartree (1951), was added to the dialysate (V in Table 1) with stirring. After standing for 30 mins., the coloured gel fraction was centrifuged off and the supernatant (VI in Table 1) was concentrated by the aid of collodin bags and the concentrate was subjected to

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sephadex G-200 column. The eluted fraction (VII in Table 1) showing protease activity was concentrated and re chromatographed by the same procedure.

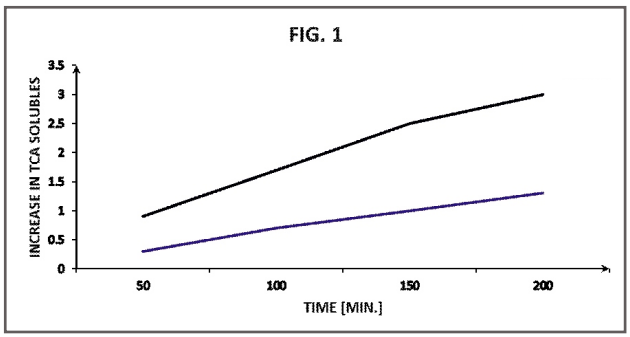
The eluted fractions were concentrated by collodin bags and used as a protease sample (VIII in Table 1) total and specific activities of protease in each step are displayed in Table 1. Under the purification procedure, protein content was determined by microkjeldahl method of Snell and Snell (1955).

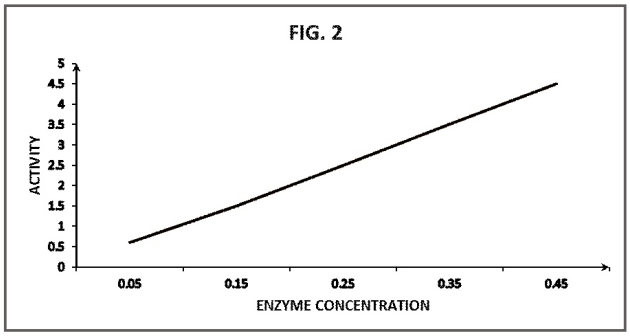
Determination of Protease Activity

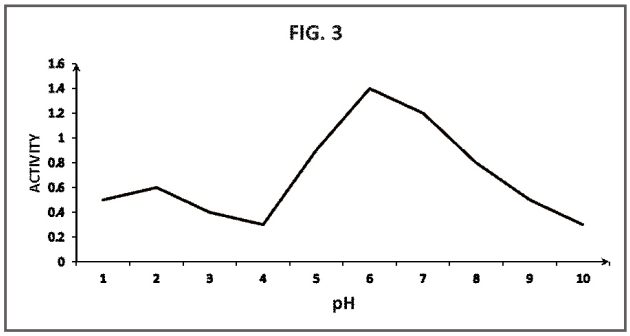
The protease activity was determined as per method of Hagiwara (1954). A volume of enzyme solution was incubated with 4 volumes of 0.6% casein in 0.1M Mcilvaine buffer (pH 5.5) for one hour at 40^{0}C in presence of 4mM cysteine. The reaction was stopped by adding tri chloro acetic acid. The protease activity was expressed in terms of increase in Folin number of the supernatant (Lowry et al.1951).

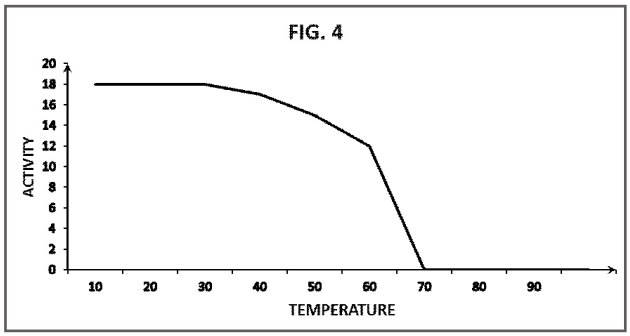
Determination of Glycine Betaine

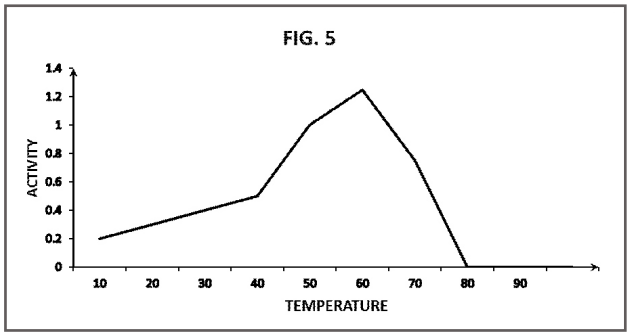
It was done by direct reflectance densitometry as described by Radecka et al., (1971) and standardized by Rathor, (2006) in this laboratory.











RESULTS AND DISCUSSION

The data displayed in Fig. 1 revealed that the action of protease enzyme with casein at 40^{0}C and 50^{0}C gradually decreased with time, while it increased by the amount of TCA soluble compounds produced after 1hr of incubation with casein. The effect of enzyme concentration on the hydrolysis under standard assay conditions is disinterred in Fig. 2. It is exhumed from the curve that a more or less linear relationship existed between the amount of enzyme concentration range examined.

The effect of pH on the protease activity was shown in Fig. 3. This reveals that the activity of protease was maximum at pH 6 and decreased abruptly on the acidic side and relatively less abruptly on the alkaline side. It is interesting to note that below pH 4.0 the protease activity was not assumed accurate due to very feeble casein

solubility. As shown in Fig. 4, the protease enzyme becomes inactive partially at temperature above 40^0C and completely inactive at about 70^0C.

As is obvious from the curve in Fig. 5, the impact of incubation temperature was also found remarkable. The fastest hydrolysis took place at 60^0C. The rate of hydrolysis got reduced at more than 50^0C, when the incubation period was prolonged. The protease activity was recorded fully diminished at 70^0C and the substrate retained as such as it was at 40^0C. This shows that the enzyme got stabilized by casein substrate.

Our results presented in Table 2 revealed that the catabolism of glycine betaine-a quaternary ammonium compound, also synthesized endogenously, could perhaps be blocked. The substantial amount of glycine betaine maintained in lucerne plants (all eight fractions) can be ascribed to restore the protein contents and protease activity successfully. Thus, it may be suggested that the glycine betaine acts as a combatment measure of sodium chloride stress.

Table 1

Protein, Total Protease Activity and Specific Protease Activity in different eight fractions under sodium chloride stress. (Values are means of three replicates)

Fractions	Protein (mgs)	Total protease activity (units × 10^2)	Specific protease activity (units/mg protein)
I	42000	1490	3.05
II	16221	793	4.80
III	11430	611	5.03
IV	4581	214	5.24
V	315.5	98	27.35
VI	155.2	71	41.35
VII	34.12	49	141.92
VIII	18.25	41	196.73

Table 2

Protein, Total Protease Activity and Specific Protease Activity in relation to glycine betaine in different eight fractions under sodium chloride stress (Values are means of three replicates)

	Fractions							
Enzyme Activity & QACs	I	II	III	IV	V	VI	VII	VIII
Proteins	42640	16453	11801	4903	360.7	159.9	35.9	19.31
Glycine betaine	64.85	64.89	64.72	65.10	64.91	63.71	63.92	64.11
Total Protease Activity	1542	811	632	228	105	83.1	51.2	42.3
Glycine betaine	68.16	68.7	6832	67.49	678	66.3	67.5	67.63
Specific Protease Activity	3.17	4.98	5.29	5.31	28.31	42.1	147.2	205.4
Glycine betaine	71.39	69.32	69.3	69.9	69.99	70.5	70.6	70.93

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