SALINITY INDUCED CHANGES IN KETO ACIDS, AMINO ACIDS AND ENZYMES OF TRANSAMINATION SYSTEM IN PIGEON PEA (*Cajanus indicus* SPRENG) AND GINGELLEY (*Sesamum indicum* L.) LEAVES[1] /

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Resumen

Se determinó el nivel de cetoácidos, aminoácidos y la actividad de las enzimas amino transferencias (azpartato y alanina) y la deshidragenasa (glutamato) en hojas de Cajanus indicus *y* Sesamun indicum, *sometidas a condiciones de salinidad con NaCl. Se encontró una acumulación de cetoácidos como el ácido oxaloacético y el ácido fosfoenol pirúvico bajo condiciones de salinidad, mientras que los ácidos α cetoglutárico y pirúvico se acumularon en el testigo. Se notó un marcado aumento en el contenido de ácido glutámico, glutamina, ácido aspártico, asparagina, glicina/serina, prolina y alanina con el aumento de la salinidad. La baja en concentración de algunos cetoácidos como el ácido α cetoglutárico, piruvato y oxaloacetato con el aumento paralelo de glutamato alanina y ácido aspártico se atribuyen al aumento de actividad de la deshidrogenasa glutamato, la amino transferasa alanina y la amino transferasa aspartato, respectivamente. Se discute la importancia de estos cambios en relación al mecanismo de adaptación de estas plantas a condiciones de salinidad.*

Introduction

Keto acids are important intermediary metabolites which provide carbon skeletons for the synthesis of amino acids and proteins (2). Earlier reports refer essentially to the occurrence of these metabolites in different plant parts (14, 17) and their role in different plant fractions (22). Although relative changes in amino acids and proteins with growth and development have been studied in detail, reports on changes in keto acids and amino acids in maturing leaves are very few. Salt induced changes in keto acid and amino acid content have been shown in pea and corn sprouts (18) and groundnut leaves (7). Studies on enzymes of transamination system under saline conditions received inadequate attention. The present study, therefore, has been undertaken to find out the changes in keto acids, amino acids and enzymes of the transamination system in pigeon pea and gingelley during progressive maturation of the leaves under NaCl salinity

Materials and methods

Pigeon pea (*Cajanus indicus* Spreng Var. LRG-30) and gingelley (*Sesamum indicum* L. Var TMV-1) were screened for tolerance to varying levels of salinity ranging from 0.1% to 0.6% NaCl in the soil. A salinity level of 0.4% has been selected in the present study at which pigeon pea was tolerant and gingelley was susceptible. The plants were raised in 18 centimeter diameter carthenware pots containing soil and manure in the ratio of 3:1. The plants were thinned to 3 plants per plot before applying the salt treatment. Salt treatment was given at two stages of growth, 15 and 30 days after sowing. The salt content of the soil was raised to 0.4% by adding NaCl solution on air dry weight basis of the soil.

The first formed trifoliate leaf from pigeon pea and first pair of leaves from gingelley were collected at the following stages for analyses.

Stage 1: 7 days after first NaCl treatment (when the leaves showed full opening);

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Stage 2: 15 days after first NaCl treatment (active period of growth);

Stage 3: 7 days after second NaCl treatment (maturation phase); and

Stage 4: 15 days after second NaCl treatment (initiation of senescence). The physiological changes induced by salinity showed recovery symptoms at stage 2 (30 days after sowing), and a second treatment was therefore given to maintain the same level of inhibition and to elicit a clear response from the plants (6).

Amino acids were extracted (3) and estimated (5) by following standard procedures. Extraction, chromatographic analysis and quantitative estimation of keto acids were done according to Mukherjee (16). Preparation of enzyme extract i e.; Aspartate amino transferase (Glutamate oxaloacetate transaminase – GOT – EC 2.6.1.1) and Alanine amino transferase (Glutamate pyruvate transaminase – GPT – EC 2 6 1 2) (11) and their assay (19) were carried out on all the stages. Glutamate dehydrogenase (GDH – EC 1 4 1 3) activity was determined by the method of Joy (13). Protein content of these enzyme extracts was estimated according to Lowry *et al* (15). Triplicate samples were analysed for each experiment.

Results

Levels of different amino acids present in pigeon pea and gingelley leaves are given in Tables 1 and 2 respectively. In gingelley glutamine and hydroxyproline were found to be absent (Table 2). Amino acids such as asparagine, aspartic acid, glycine/serine, glutamic acid, glutamine (in pigeon pea), alanine and proline were found to be accumulating both in pigeon pea and gingelley under salinity. Hydroxyproline also showed accumulation in pigeon pea under salinity. Of all the amino acids, glutamic acid showed greater accumulation in pigeon pea leaves under salinity.

γ-aminobutyric acid, valine phenylalanine and threonine showed a decrease under salinity in both species. Leucine/iso-leucine showed a decrease in pigeon pea and increase in gingelley under salinity.

Tables 3 and 4 indicate the levels of keto acids in pigeon pea and gingelley, respectively. Oxaloacetic acid was found to be absent in gingelley but present in pigeon pea. Keto acids such as phosphoenol pyruvic acid (PEP) and oxaloacetic acid (OAA) showed a high degree of accumulation in both species under salinity on all the stages of growth. Pyruvic acid (Pyr) showed a slight degree of accumulation up to stage 2 and a decrease up to stage 4 in both species under salinity. α-ketoglutaric acid (α-KGA) showed a slight degree of increase at stage 1 and decrease stages 2, 3 and 4 in pigeon pea and gingelley under salinity. Glyoxylic acid (Gly) showed a slight degree of accumulation at stage 1 followed by a decline up to stage 4 in gingelley and a decrease from stage 1 to stage 4 in pigeon pea under salinity. Oxalosuccinic acid and two unidentified spots were found to be present in gingelley only.

All the three enzymes, GPT, GOT and GDH showed increased activity in both the plants under salinity

Table 1. Levels of amino acids in pigeon pea leaves under control and NaCl salinity (μg/g fresh weight).

Amino acid	Stage 1		Stage 2		Stage 3		Stage 4	
	Control	Salinized	Control	Salinized	Control	Salinized	Control	Salinized
Asparagine	45	136	56	157	78	198	96	214
Aspartic acid	125	254	146	283	177	318	192	326
Glycine/Serine	265	284	288	312	325	335	348	352
Hydroxyproline	36	68	49	89	75	112	58	136
Arginine	57	75	74	89	88	109	72	126
Glutamic acid	256	362	294	398	305	427	326	459
Alanine	165	224	188	258	196	270	224	296
γ-aminobutyric acid	112	98	132	115	147	126	128	103
Proline	46	158	58	176	74	197	86	224
Valine	64	51	78	63	96	78	121	90
Phenylalanine	65	74	79	89	82	98	76	108
Glutamine	98	136	114	168	129	193	108	216
Threonine	47	59	58	61	70	77	108	60
Leucine/iso-leucine	87	78	98	83	112	85	104	94

Table 2. Levels of amino acids in gingelley leaves under control and NaCl saline conditions (μg/g fresh weight).

Amino acid	Stage 1		Stage 2		Stage 3		Stage 4	
	Control	Salinized	Control	Salinized	Control	Salinized	Control	Salinized
Asparagine	45	64	57	89	74	108	89	126
Aspartic acid	98	128	114	152	126	179	133	195
Glycine/Serine	165	179	180	216	212	235	224	245
Arginine	108	137	112	159	147	184	136	193
Glutamic acid	102	154	124	183	136	198	128	216
Alanine	84	142	96	158	119	172	106	164
γ-aminobutyric acid	36	24	47	36	52	49	64	37
Proline	TRACE	46	TRACE	89	27	124	38	136
Valine	64	58	89	62	97	86	108	74
Phenylalanine	64	72	89	96	94	120	112	137
Threonine	57	59	65	74	76	84	83	92
Leucine/iso-leucine	65	84	93	93	86	113	97	132

Table 3. Levels of keto acids in pigeon pea leaves under control and NaCl conditions (mg/g fresh weight).

Keto acid	Stage 1		Stage 2		Stage 3		Stage 4	
	Control	Salinized	Control	Salinized	Control	Salinized	Control	Salinized
Phosphoenolpyruvic acid	–	1 90	0 45	2 20	0 48	2 50	0.49	2 35
Pyruvic acid	2 60	3 00	2 80	3 30	3 20	2 98	3 05	2 74
Oxaloacetic acid	1 00	2 35	1 45	2 96	1 62	3 45	1 50	3 25
Glyoxylic acid	1 35	1 30	1.40	1 16	1.52	0 95	1 25	0 56
α-ketoglutaric acid	0 58	0 95	0 95	0 72	1.25	0 56	1.45	0 45

Table 4. Levels of keto acids in gingelley leaves under control and NaCl saline conditions (mg/g fresh weight).

Keto acid	Stage 1		Stage 2		Stage 3		Stage 4	
	Control	Salinized	Control	Salinized	Control	Salinized	Control	Salinized
Phosphoenolpyruvic acid	0 28	0 56	0 30	0 85	0 46	1 15	0 58	1 06
Pyruvic acid	1 98	2 50	2 10	2 20	2 25	1 98	2 46	1 60
Oxaloacetic acid	–	0 30	–	0 56	–	0 95	–	0 72
Glyoxylic acid	0 95	1 30	1 10	1 05	1 20	0 80	0 80	0 65
α-ketoglutaric acid	0 36	0 45	0 59	0 39	0 98	0 30	1 15	0 21
Oxalosuccinic acid	1 20	1 10	1 35	1 05	1 20	0 96	0 95	0 64
Unidentified 1	–	0 50	0 15	0 62	0 20	0 75	0 36	0 45
Unidentified 2	0 26	0 37	0 48	0 69	0 64	0 96	0 54	1 10

(Figura 1). GPT showed an increase from stage 1 to stage 3 followed by a decrease at stage 4 and a decrease from stage 1 to stage 3 followed by an increase at stage 4 in pigeon pea under control and salinized conditions, respectively. An increase from stage 1 to stage 4 and an increase from stage 1 to stage 3 and a decline at stage 4 was observed in gingelley under control and salinized conditions, respectively.

GOT showed a continuous increase from stage 1 to stage 4 and a slight decrease from stage 2 to stage 3 followed by an increase at stage 4 in pigeon pea under control and saline conditions, respectively. GOT showed a similar trend as GPT in gingelley both under control and salinized conditions.

GDH showed a decrease from stage 1 to stage 3 with an increase at stage 4 in pigeon pea; and a decrease from stage 1 to stage 2 followed by an increase up to stage 4 in gingelley both under control and saline conditions.

Discussion

Keto acids represent a link between the carbohydrate and nitrate metabolism explaining the great importance in the keto acid metabolism in plants growing under saline conditions. Strogonov (20) emphasised that keto acids play a protective role in saline habitats in addition to the metabolic functions. These protective functions consist in binding the excess ions absorbed by the plant and in maintaining the electrical neutrality of the cells and finally neutralising the basic compounds.

Generally, in plants growing under saline conditions ammonia tends to accumulate enormously which is said to be toxic to the plant growth (18). Keto acids play a pivotol role by participating in amination and transamination reactions, thus detoxifying ammonia to form amino acids. Generally, the process of detoxification proceeds via the amination and transamination system (7):

KETO ACIDS ⇌ DICARBOXYLIC AMINO ACIDS (GLUTAMIC AND ASPARTIC ACIDS) ⇌ AMIDES (GLUTAMINE AND ASPARAGINE).

From the present study it is presumed that α-KGA, Pyr and OAA in salinized plants participate in the above reactions. In this case alanine can be formed pyruvate, glutamic acid from α-ketoglutarate and aspartic acid from oxaloacetate. Conversely, no such greater accumulation of amino acids is evident in controls. These reactions are in turn regulated by the enzymes, alanine amino transferase (GPT), glutamate dehydrogenase (GDH) and aspartate amino transferase (GOT), respectively. The enhanced activity of these enzymes under salinity results in the formation of higher amounts of amino acids and amides such as alanine, glutamate, glutamine, aspartate and asparagine, there by decreasing the toxicity of ammonia. Thus the enhanced activity of

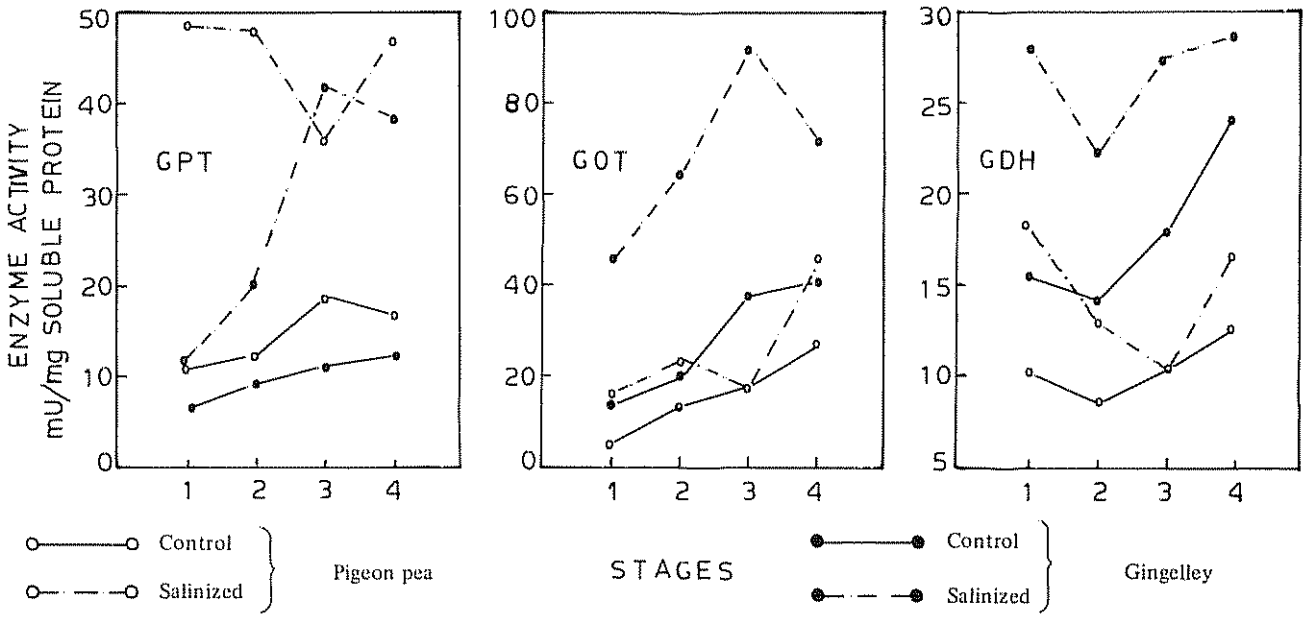

Fig 1. Activities of the enzymes Alanine amino transferase (GPT), aspartate amino transferase (GOT) and glutamate dehydrogenase (GDH) in pigeon pea and gingelley leaves under NaCl salinity.

GPT, GDH and GOT which results in the conversion of pyruvate, α-ketoglutarate and oxaloacetate to amino acids in the presence of accumulated ammonia (20) may possibly play a protective role under saline conditions. The results of the present study are in conformity with those of Joshi (12) and Gururaja Rao and Rajeswara Rao (9), who observed high GPT, GDH and GOT activities in marine algaem mangroves and a C_3 plant, wild rice (*Oryza sativa* L. Var. Kala Rata) and peanut leaves, respectively. The low amounts of PEP and high amounts of OAA in the initial stages of leaf growth indicate that PEP → OAA pathway was actively operating during the early stages. In control plants as the leaves mature the transamination reactions utilizing the keto acids for the synthesis of amino acids is affected and become sluggish.

The keto acids like OAA and PEP are formed during respiration as well as photosynthesis. But plants like pigeon pea and gingelley, which fix carbon by C_3 pathway, do not synthesise them under normal conditions during photosynthesis. The accumulation of these acids in salinized plants envisages that these metabolites are synthesised during an altered pathway of photosynthesis. Salinity induced shift from C_3 to C_4 pathway (aspartate type) was reported by Joshi (12) in marine algae, mangroves and a C_3 plant, *Oryza sativa* Var. Kala Rata. The conversion of OAA to aspartate was found to be stimulated by NaCl salinity in these plants. Both, pigeon pea and gingelley also showed high rates of $^{14}CO_2$ incorporation into OAA, aspartate, and less incorporation into sugars and sugar phosphates (8). Generally in a low salt environment the plants show C_3 metabolism and with the increase in salinity the assimilation shifts to C_4 dicarboxylic acid pathway as in the case of *Aleuropus litoralis*, a halophytic grass (4) and *Cakile maritima* (1). But in *Mesembryanthemum crystallinum* (24) and *Portulacaria afra* (21), the shift is towards crassulacean acid metabolism (CAM) and thus increasing the organic acid content for osmoregulation. It was also shown that watering of *M. crystallinum* plants with NaCl solutions resulted in high Na^+ than Cl^- in the leaves, which indicates that Na^+ should facilitate in changing the metabolism. The occurrence of low amounts of OAA and PEP at stage 4 in the salinized plants may be due to the reduced rates of synthesis as the leaves showed the initiation of senescence. In the present study the occurrence of C_4 cycle acids under NaCl salinity was unaccompanied by Kranz type of leaf anatomy. On the other hand the leaves of salt-stressed plants developed succulence (6). Salinity induced succulence of leaves was shown in *Phaseolus vulgaris* (23) and in *Cajanus indicus* and *Cyamopsis tetragonoloba* (10). Thus, the succulent nature of the leaves and the occurrence of PEP and OAA in higher amounts in salinized pigeon pea and gingelley leaves can be interpreted in terms of a shift from C_3 to weak CAM type, as reported earlier (7).

Literature cited

1. BEER, S. *et al.* Salt stimulated phosphoenolpyruvate carboxylase in *Cakile maritima*. Physiologia Plantarum 34:293-295, 1975.

2. COSSINS, E. A. and SINHA, S. K. Occurrence and properties of L-amino acid: 2-Glyoxylate aminotransferase in plants. Canadian Journal of Biochemistry 43:495-506, 1965.

3. DAS CHAUDHURY, T. K. *et al.* A note on the estimation of free amino acids, sugars and organic acids from gummy and resinous plant material. Indian Journal of Plant Physiology 10:196-201, 1967.

4. DOWNTON, W. J. S. and TOROKFALVY, E. Effect of sodium chloride on the photosynthesis of *Aleuropus litoralis*, a halophytic grass. Zeitschrift fur Pflanzenphysiologie 75:143-150, 1975.

5. GIRI, K. V. *et al.* Some factors influencing the determination of amino acids separated by circular paper chromatography. Annals of Chemistry 24:1677-1678, 1952.

6. GURURAJA RAO, G. Physiological studies on the influence of sodium chloride salinity on leaf metabolism in pigeon pea (*Cajanus indicus* Spreng) and gingelley (*Sesamum indicum* L.). Ph D. Thesis, Sri Venkateswara University, Tirupati, India, 1980.

7. GURURAJA RAO, G. and RAJESWARA RAO, G. Sodium chloride salinity induced changes in keto acids and amino acids in peanut (*Arachis hypogaea* L.) leaves. Indian Journal of Plant Physiology 22:121-126, 1979.

8. GURURAJA RAO, G. and RAJESWARA RAO, G. $^{14}CO_2$ incorporation studies in pigeon pea and gingelley under salt – stress. Journal of Nuclear Agriculture and Biology, 1981. (in press).

9. GURURAJA RAO, G. *et al.* Salinity induced changes in the activities of aspartate and alanine amino transferases and glutamate dehydrogenase in peanut leaves. Indian

Journal of Experimental Biology 19:771-772, 1981.

10. GURURAJA RAO, G *et al.* Salt induced anatomical changes in the leaves of pigeon pea (*Cajanus indicus* Spreng) and cluster beans (*Cyamopsis tetragonoloba* (L.) Taub) Proceedings of Indian Academy of Sciences 88:293-301, 1979.

11. HEDLEY, C. L. and STODDART, J. L. Light stimulation of alanine amino transferase activity in dark grown leaves of *Lolium tomentulum* L. as related to chlorphyll formation. Plants (Berlin) 100:309-324, 1971.

12. JOSHI, G. V. Photosynthesis under saline conditions. PL-480 Project Final Report, Shivaji University, Kolhapur, India, 1976.

13. JOY, K. Nitrogen metabolism of *Lemna minor*. II. Enzymes of nitrate assimilation and some aspects of their regulation. Plant Physiology 44:849-853, 1969.

14. KRUPKA, R. M. and TOWERS, G. H. N. Keto acids. Botanical Review 25:108-120, 1959.

15. LOWRY, O. H. *et al.* Protein measurement with Folin phenol reagent. Journal of Biological Chemistry 93:265-276, 1951.

16. MUKHERJEE, D. Keto acids and amino acids in *Delonix regia* flowers. Phytochemistry 14:1915-1918, 1975.

17. MUKHERJEE, D. and LALORAYA, M. M. Keto acids and free amino acids during leaf growth in *Bauhinia purpurea* L. Experientia 33:305-306, 1977.

18. PRIKHODKO, L. S. Hydroxy and keto acid metabolism in pea and corn sprouts under substrate salinization. Soviet Plant Physiology 15:674-680, 1968.

19. REITMAN, S. and FRANKEL, S. Determination of glutamic pyruvic transaminase in serum. American Journal of Clinical Pathology 28:56-64, 1967.

20. STROGONOV, B. P. Structure and function of plant cells in saline habitats. New Trends in the study of salt tolerance. Israel Programme for Scientific Translations, Jerusalem. 1970.

21. TING, I. and HANSCOM, Z. Induction of acid metabolism in *Portulacaria afra*. Plant Physiology 59:511-514, 1977.

22. WEBB, J. A. and FOWDEN, L. Changes in oxoacid concentrations during the growth of groundnut seedlings. Biochemical Journal 61:1-3, 1955.

23. WIGNARAJAH, K. *et al.* The effect of salinity on the growth of *Phaseolus vulgaris* L. Anatomical changes in the trifoliate leaf. Annals of Botany 39:1029-1038, 1975.

24. WINTER, K. Zum problem der Ausailding des Crassulacee saurestoffweschsels bei *Mesembryanthemum crystallinum* under NaCl EinfluB. Planta (Berlin) 109:135-145, 1973.