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TRANSAMINATION OF GLUTAMIC ACID DURING GERMINATION, GROWTH AND SEED DEVELOPMENT IN BENGAL GRAM

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Abstract—The activity of glutamic acid aminotransferase with respect to six keto acids was studied in dialysed extracts obtained from germinating seeds, leaves of plants at seedling, preanthesis and postanthesis stages and the developing seeds of Bengal gram. There was a considerable amount of non-enzymic aminotransferase activity which changed at different stages of growth. The efficiency of glutamic acid to transfer amino group to various keto acids also varied in two different varieties in relation to growth and development. The possibility of the existence of either many aminotransferases or of several isozymes is discussed.

IN HIGHER plants glutamic acid still seems to be the main port of entry of nitrogen[1] though there seems some possibility of the synthesis of alanine, aspartic acid and glycine by their respective dehydrogenases.[2] However, the latter claims have yet to be critically examined. Consequently it can be assumed that the transfer of amino group from glutamic acid to different keto acids should play a significant role in the synthesis of other amino acids. The present study was aimed at examining the efficiency of glutamic acid to transfer its amino group to six different oxo acids at different stages of growth and development in Bengal gram (*Cicer arientinum*). In earlier studies it was shown that the transamination activity varied at the species level;[3-5] therefore, it was considered of interest to extend these studies at the varietal level.

RESULTS

Six systems responsible for the synthesis of aspartic acid, alanine, tyrosine, serine, glycine and leucine were studied during germination, seedling growth, pre-anthesis, post-anthesis and seed development stages. The reaction mixture minus keto acid was used as a control. Further, the boiled enzyme preparation was used to determine whether the reaction was non-enzymic. At two stages, the seedling and pre-anthesis, the aminotransferase activity in boiled extracts was more than in the control lacking keto acid. Therefore, it would appear that at certain stages nonenzymic catalysis could account for considerable amount of transamination.

Seed germination. All the six systems were active in the transamination of glutamic acid during germination. The activity of extracts obtained from two varieties was almost the same for all the systems except hydroxyphenylpyruvate and glyoxylate. In the variety BGS 1 synthesis of tyrosine and glycine was 25 and 50 per cent more respectively as compared to

[1] L. FOWDEN, *Ann. Rev. Pl. Physiol.* **18**, 85 (1967).
[2] W. L. KRETOVICH, *Ann. Rev. Pl. Physiol.* **16**, 141 (1965).
[3] E. A. COSSINS and S. K. SINHA, *Can. J. Biochem.* **43**, 495 (1965).
[4] S. K. SINHA, *Indian J. Biochem.* **3**, 14 (1966).
[5] S. K. SINHA, G. S. SIROHI and Y. P. ABROL, *Indian J. Exptl Biol.* **5**, 190 (1967).

TABLE 1. GLUTAMIC ACID AMINOTRANSFERASE ACTIVITY IN GERMINATING SEEDS OF BENGAL GRAM

Keto acid added	Variety NP 100 Counts/ min/mg protein	% of control	Variety BGS 1 Counts/ min/mg protein	% of control
None	1190	100	1180	100
Boiled enzyme + pyruvate	1073	90	1146	96
Pyruvate	1860	156	2196	185
Oxaloacetate	2227	187	2817	237
Hydroxyphenylpyruvate	3500	294	4550	384
Hydroxypyruvate	1760	147	1540	129
Glyoxylate	3500	294	5714	482
Oxoisocaproate	1570	132	1610	136

Complete reaction mixture contained 0·2 μc glutamic acid-^{14}C having specific activity of 11·5/μmole, 50 μg pyridoxal phosphate and 0 5 ml enzyme preparation. Additions of 10 μmoles of keto acids were made as indicated. The total volume of the reaction mixture in phosphate buffer was 2 ml and the incubation period was 30 min at 35°.

the variety NP 100. The ability of extracts to synthesize serine and leucine was relatively poor in both the varieties (Table 1).

Seedling and pre-anthesis stage. The specific activity of all the systems enhanced several-fold in the leaves of 6-day-old seedling and of plants before anthesis compared to the activity observed in germinating seeds. A very distinctive feature was of strong stimulation in the transfer of amino group to glyoxylate and later on to oxaloacetate in the variety NP 100 (Table 2). The transfer of amino group to pyruvate, hydroxypyruvate, hydroxyphenylpyruvate and oxoisocaproate was enhanced 2–4-fold. However, in the variety BGS 1 the extent of stimulation was less for glutamate–glyoxylate system as well as other systems. A reduction in the specific activity was observed for glutamate–hydroxyphenylpyruvate system. At the preanthesis stage glutamate–glyoxylate system was much less efficient in BGS 1.

Post-anthesis stage. After anthesis the aminotransferase activity in leaf extracts rose sharply. The specific activity in the minus keto acid check was 9720 counts/min/mg protein in the variety NP 100 and 10260 counts/min/mg protein in BGS 1 (Table 3). Even the boiled preparations had very high values of 7690 counts/min/mg protein and 7200 counts/min/mg

TABLE 2. GLUTAMIC ACID AMINOTRANSFERASE ACTIVITY IN THE LEAVES OF YOUNG SEEDLINGS

Keto acid added	Variety NP 100 Counts/ min/mg protein	% of control	Variety BGS 1 Counts/ min/mg protein	% of control
None	2990	100	2470	100
Boiled enzyme + pyruvate	4380	146	2860	115
Pyruvate	6610	221	5590	226
Oxaloacetate	6810	227	5840	236
Hydroxyphenylpyruvate	6790	227	3670	148
Hydroxypyruvate	5140	171	3290	133
Glyoxylate	22,500	752	12,050	487
Oxoisocaproate	7020	234	3630	146

Details of the complete reaction mixture and incubation are the same as given in Table 1.

TABLE 3. GLUTAMIC ACID AMINOTRANSFERASE ACTIVITY IN THE LEAVES OF PLANTS BEFORE ANTHESIS

Keto acid added	Variety NP 100 Counts/ min/mg protein	% of control	Variety BGS 1 Counts/ min/mg protein	% of control
None	2030	100	2770	100
Boiled enzyme + pyruvate	4210	207	3710	134
Pyruvate	6820	335	4730	171
Oxaloacetate	14,700	723	4540	164
Hydroxyphenylpyruvate	9830	483	4260	154
Hydroxypyruvate	9430	463	5640	204
Glyoxylate	11,630	572	5690	205
Oxoisocaproate	5960	293	5080	183

Details of the reaction mixture and incubation are given in Table 1.

protein in NP 100 and BGS 1 respectively. This shows that nonenzymic activity accounted for considerable amino group transfer activity from glutamic acid. However, the relative activity of different systems with respect to the minus keto acid check again showed variation. Three systems viz. glutamate–oxaloacetate, glutamate–pyruvate and glutamate–glyoxylate were more active than the other three systems. The efficiency of all the systems varied in two varieties. In NP 100 glutamate–oxoisocaproate was the least efficient while in BGS 1 glutamate–hydroxypyruvate and glutamate–hydroxyphenylpyruvate were poorest in activity.

Developing seeds. The aminotransferase activity of the extracts prepared from developing seeds 13 days after anthesis was less than what was observed in leaves after anthesis. However, the activity was more when compared to other stages of growth. In developing seeds the glutamate–oxaloacetate aminotransferase showed the highest activity in NP 100 while in the variety BGS 1 glutamate–glyoxylate aminotransferase was most active. The transfer of amino group to hydroxyphenylpyruvate was less than the minus keto acid value.

TABLE 4. GLUTAMIC ACID AMINOTRANSFERASE ACTIVITY IN THE LEAVES OF PLANTS AFTER ANTHESIS

Keto acid added	Variety NP 100 Counts/ min/mg protein	% of control	Variety BGS 1 Counts/ min/mg protein	% of control
None	9720	100	10,260	100
Boiled enzyme + pyruvate	7690	79	7200	70
Pyruvate	23,800	245	22,420	218
Oxaloacetate	28,380	292	21,300	208
Hydroxyphenylpyruvate	14,670	151	13,960	136
Hydroxypyruvate	17,280	178	14,270	139
Glyoxylate	25,090	258	19,500	190
Oxoisocaproate	13,670	141	16,060	157

Details of the reaction mixture and incubation are given in Table 1.

TABLE 5. GLUTAMIC ACID AMINOTRANSFERASE ACTIVITY IN THE DEVELOPING SEEDS, 13 DAYS AFTER ANTHESIS

Keto acid added	Variety NP 100 Counts/ min/mg protein	% of control	Variety BGS 1 Counts/ min/mg protein	% of control
None	5950	100	11,520	100
Boiled enzyme + pyruvate	5140	86	4020	35
Pyruvate	10,340	174	16,920	147
Hydroxyphenylpyruvate	4730	80	11,230	97
Oxaloacetate	13,590	229	18,170	158
Hydroxypyruvate	10,910	184	21,060	183
Glyoxylate	9200	155	23,800	207
Oxoisocaproate	11,360	191	20,130	175

Details of the reaction mixture and incubation are given in Table 1.

DISCUSSION

The present study brings out three facts; (I) glutamic acid can transfer amino group to pyruvate, oxaloacetate, glyoxylate, hydroxypyruvate, hydroxyphenylpyruvate and oxoisocaproate at all stages of growth and development in Bengal gram. Furthermore, the ability of extracts to transfer amino group to different keto acids varies at different stages of plant growth. For example, during germination the amino group was transferred more efficiently to hydroxyphenylpyruvate and glyoxylate but afterwards pyruvate and oxaloacetate were better acceptors, (II) the aminotransferase activity of the same system differs at the varietal level, (III) there appears a possibility of fairly high rate of nonenzymic transamination and also of exchange reactions.

The first conclusion is important from the point of view of the nature of aminotransferase enzyme. It is now known that a number of isozymes of glutamate–oxaloacetate exist in some plants[6] but we know very little of the specificity of these isozymes to different keto acids. This leaves open the question whether one protein carries out all the reactions with different degrees of efficiency or there are different proteins for different systems. Cossins and Sinha[7] obtained some evidence showing existence of different proteins for glutamate–pyruvate and glutamate–oxaloacetate systems, using ammonium sulphate precipitation method. More recently Brack *et al.*[8] employing several criteria have shown the presence of serine–glyoxylate and alanine–glyoxylate aminotransferases catalysed by different proteins in oat leaves.

Changes in proteins during growth and differentiation have been shown by Barber and Steward.[9] Some proteins make their appearance only at the time of flowering as in the bulbous plant *Tulipa alba*. It was reported by Sinha[4] that the glyoxylate aminotransferase activity from the vegetative and reproductive mycelia varied in *Cyathus*. The activity in the latter was almost double compared to the former. Sengupta[10] recently observed changes in glyoxylate aminotransferase during the growth of wheat and the maximum activity was observed at the time of flowering. In the present study, also, changes in aminotransferase

[6] J. G. SCANDALIOS, *Biochem. Genetics* **3**, 37 (1969).
[7] E. A. COSSINS and S. K. SINHA, *J. Exptl Bot.* **18**, 215 (1967).
[8] B. L. BRACK, D. A. WILKINSON and J. KING, *Can. J. Biochem.* **48**, 486 (1970).
[9] J. T. BARBER and F. C. STEWARD, *Development Biol.* **17**, 326 (1968).
[10] U. K. SENGUPTA, Ph.D. Thesis, Indian Agricultural Research Institute, Delhi (1968).

activity were observed and the maximum activity was obtained after anthesis. The stimulation in the activity of these systems is understandable because of the increased requirement of various amino acids for protein synthesis and of some constituents for which some amino acids serve as precursors, for example glycine. However, it is not clear whether this stimulation is due to the synthesis of different isozymes or activation of some inactive proteins, or some other changes making a unifunctional system function like a multifunctional system. Detailed studies in this direction are necessary.

In addition, some amino acids such as serine can be synthesized through a mechanism other than transamination of hydroxypyruvate.[11–13] It should be interesting to know which particular mechanism is more efficient in serine biosynthesis in relation to growth and differentiation.

EXPERIMENTAL

The seed material of NP 100 and BGS 1 was obtained from the Genetics Division of the Institute. Plants were raised under natural conditions. Leaves of comparable age were harvested at all stages of growth. Flowers were tagged to obtain developing seeds of a definite age.

Plant material 0·5–1 g was homogenized in 0·1 M phosphate buffer of pH 7·6. It was then passed through two layers of muslin and centrifuged at 10,000 *g* for 10 min. The supernatant was dialysed against H_2O until free of soluble amino acids. After dialysis this extract was used as source of enzyme.

The assay of glutamate aminotransferase was done following the method of Cossins and Sinha.[3] Glutamic acid-^{14}C was used as the donor amino acid and the formation of ketoglutarate was a measure of amino-transferase activity. The incubation period was 30 min at 35°. It was noted earlier that the equilibrium is reached by this time.[3] The identity of the keto acid was determined as described earlier.[3] Protein determination was done by precipitating it with 10% TCA and followed by digestion and Nesselerization.

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[11] A. P. Wilkinson and D. D. Davies, *J. Exptl Bot* **11**, 296 (1960).
[12] S. K. Sinha and E. A. Cossins, *Biochem J.* **93**, 27 (1964).
[13] E. A. Cossins and S. K. Sinha, *Can. J. Biochem.* **43**, 685 (1965).