Phytochemistry, 1979, Vol. 18, pp. 409–410 © Pergamon Press Ltd. Printed in England. 0031-9422/79/0301-0409 $02.00/0

AMINO ACID COMPOSITION OF VASCULAR SAP OF MAIZE EAR PEDUNCLE

P. ARRUDA and W. J. DA SILVA

Departamento de Genética e Evolução, Instituto de Biologia, Universidade Estadual de Campinas, C.P. 1170, Campinas, São Paulo, Brazil

(*Revised received* 14 *August* 1978)

Key Word Index—*Zea mays*; Gramineae; maize; amino acids; nitrogen transport.

Abstract—The amino acid composition of the vascular sap of a high lysine maize mutant was determined during kernel development. With the exception of proline and cystine, all amino acids that occur in the endosperm were found in the vascular sap of the ear peduncle. Glutamine is the major amino acid transported to the endosperm varying from 30.6 to 20.6 μmol% at 7 and 42 days after pollination, respectively. Aspartic acid, the second most important nitrogen form translocated to the seeds, was *ca* 10 μmol% during kernel filling. Glutamine and arginine content decreased with maturity, while valine, methionine, isoleucine, leucine, tyrosine and phenylalanine increased with kernel development. The remaining N forms were constant during endosperm growth.

INTRODUCTION

The nitrogen (N) forms translocated to the seeds associated with corresponding metabolic enzymes in the endosperm, have become a subject of great interest in recent years. Evidence for amino acid translocation through the vascular system has been reported in some species [1–3]. In *Lupinus* [3] a high content of asparagine in the sap of the xylem was highly correlated with asparaginase activity in the developing seeds. It has been also demonstrated [3–5], that asparagine and glutamine are the major N compounds entering *Lupinus* seeds. However, great differences may occur in the concentration of these two amides in different plant species [1, 3].

In maize, a high glutamate synthase activity during seed development [6] was associated with high levels of free glutamine in the endosperm [7, 8]. However, little is known about the amino acids which are translocated to maize endosperm during development. We therefore studied the amino acid composition of the vascular sap of maize ear peduncles during kernel development.

RESULTS AND DISCUSSION

The N content decreases slowly with ear maturity varying from 1.7 at 7 days after pollination (DAP) to 1.3 mg/ml at 42 DAP, respectively (Table 1). All amino acids

Table 1. Nitrogen content and amino acid composition of vascular sap of maize ear peduncle during ear development

Component		Days after pollination					
		7	14	21	28	35	42
Total nitrogen (mg/ml)		1.7	1.5	1.6	1.4	1.4	1.3
Amino acid (μmol/100 μmol)	Lys	4.4	4.8	4.0	5.0	4.4	2.7
	His	3.3	4.4	2.6	3.2	3.2	5.5
	NH_3	6.2	4.3	5.3	6.1	4.3	6.6
	Arg	6.3	5.5	3.8	1.8	2.4	2.1
	Trp	4.5	5.3	5.2	4.5	4.8	2.7
	Asp	9.1	12.1	12.6	7.9	10.1	9.1
	AsN	5.5	6.5	5.6	5.1	6.2	7.9
	Thr	3.9	3.5	4.1	5.7	4.1	3.7
	Ser	6.1	5.6	6.3	8.1	6.8	7.4
	Glu	1.5	1.6	3.8	3.2	1.8	1.1
	GlN	30.6	28.5	25.8	17.5	20.7	20.6
	Gly	3.0	1.5	2.4	4.8	3.1	3.1
	Ala	5.8	5.8	6.0	4.4	3.4	4.5
	Val	3.2	3.1	3.4	6.0	6.0	5.1
	Met	0.9	0.9	0.8	1.3	1.7	1.4
	Ileu	1.6	1.8	1.8	2.7	3.6	3.3
	Leu	2.0	2.3	2.3	3.2	4.3	3.9
	Tyr	1.5	2.0	3.7	8.0	8.0	7.2
	Phe	0.6	0.6	0.6	1.5	1.2	2.0
	Cys	0.0	0.0	0.0	0.0	0.0	0.0
	Pro	0.0	0.0	0.0	0.0	0.0	0.0

that occur in the endosperm proteins [8–12] are present in the peduncle vascular sap, with the exception of proline and cystine. This indicates that proline and cystine are totally synthesized in the endosperm. In general, the amino acid composition, was constant during ear development, although a slight fluctuation could be observed for some amino acids. Arginine decreased from 6.3 at 7 DAP to 2.1 μmol% at 42 DAP, whereas glutamine was reduced from 30.6 at 7 DAP to 20.6 μmol% at 42 DAP. On the other hand valine, methionine, isoleucine, leucine, tyrosine and phenylalanine showed an increase during ear development. The remaining amino acids were constant during endosperm filling.

As was previously suggested by Sodek and Silva [6], our results indicated that glutamine is the major amino acid found in the vascular sap. This is probably related to the free glutamine found in the developing endosperm [7, 8] and correlates with glutamate synthase activity in maize endosperm [6]. Aspartic acid (10 μmol%) is also an important source of N transported to the developing kernel. In contrast with findings in *Lupinus* [3], asparagine (6.1 μmol%) does not seem to be a major N form in maize vascular sap during kernel development.

EXPERIMENTAL

A double mutant sugary-1 opaque-2 (*su1o2*) maize variety was planted in October 1976 in the experimental area of the Universidade Estadual de Campinas. Plants were spaced 0.2 × 1 m providing a population density of a 50000 plants/ha. The plots in the field were fertilized on the basis of 80 kg of N, 60 kg of P_2O_5 and 30 kg of K_2O per hectare.

Ear peduncles for amino acid sap analysis were taken at 7, 14, 21, 28, 35 and 42 days after pollination in such a way as to maintain the same level of plant competition through maturity. Peduncles (10, *ca* 5 cm long) were cut from the base of the ear and vascular sap was extracted from each stage as follows: the peduncle was attached to the top of a test-tube and a vacuum (0.5 atm) was applied to extract the vascular sap. $CHCl_3$ (5 ml) was passed through the peduncle to facilitate extraction. The extract from each of the 10 peduncles was pooled and separated from the $CHCl_3$ by centrifugation at 1000 *g*. The vascular sap (*ca* 1 ml from each stage of development) was then stored at −20°. N analysis was done using the procedure described in ref. [13]. Amino acid analysis was performed in an automatic amino acid analyser, using a ligand system [14]. Individual amino acids were calculated from the total amino acid recovered. Amides were determined by difference in glutamic and aspartic acid contents before and after hydrolysis with N HCl for 3 hr at 105° [8]. Before hydrolysis, glutamine was eluted with threonine. After hydrolysis glutamine was converted into glutamic acid, allowing the determination of glutamine and threonine.

REFERENCES

1. Lorenz, H. (1976) *Plant Soil* **45**, 169.
2. Lawrence, J. M. and Grant, D. R. (1964) *Arch. Biochem. Biophys.* **104**, 73.
3. Atkins, C. A., Pate, J. S. and Sharkey, P. J. (1975) *Plant Physiol.* **56**, 807.
4. Lewis, O. A. M. (1975) *J. Exp. Botany* **26**, 361.
5. Pate, J. S., Sharkey, P. J. and Lewis, O. A. M. (1974) *Planta* **120**, 229.
6. Sodek, L. and Silva, J. W. (1977) *Plant Physiol.* **60**, 602.
7. Arruda, P., Silva, J. W. and Teixeira, J. P. F. (1978) *Phytochemistry* **17**, 1217.
8. Sodek, L. and Wilson, C. M. (1971) *J. Agric. Food Chem.* **19**, 1144.
9. Baudet, J., Mossé, J., Landry, J. and Moureaux, T. (1966) *Ann. Physiol. Vég.* **8**, 321.
10. Bressani, R. and Mertz, E. T. (1958) *Cereal Chem.* **35**, 227.
11. Landry, J. and Moureaux, T. (1970) *Bull. Soc. Chim. Biol.* **52**, 1021.
12. Mertz, E. T., Bates, L. S. and Nelson, O. E. (1965) *Science* **148**, 1741.
13. Gerhke, C. W., Wall, L. L. and Absheer, J. S. (1973) *JAOAC* **56**, 1096.
14. Perkin–Elmer, (1971) Instruction manual for ligand method by single sequential system with Model KLA-3B amino acid analyser. Norwalk, Connecticut, U.S.A.