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AMINO ACIDS IN STIGMAS OF *PENNISETUM AMERICANUM*

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Key Word Index—*Pennisetum americanum*; Gramineae; pearl millet; amino acids; asparagine.

Abstract—Free asparagine levels in stigma extracts of male-sterile pearl millet increased 4.3-fold during development. At the latest stage of development, asparagine accounted for 74% of the total free amino acids in the stigmas. Together, asparagine, glutamine and serine comprised 87% of the total free amino acids. Whole, hydrolysed, non-extracted stigmas from fertile and male-sterile pearl millet showed a similar amino acid composition but both were very different than their free amino acid pools. Aspartic and glutamic acids, glycine and alanine accounted for 46% and 50% of the total amino acids recovered in the whole, hydrolysed fertile and male-sterile stigmas, respectively. Very low levels of asparaginase and asparagine aminotransferase were detected suggesting that asparagine was not metabolized and accumulates in the stigmas.

INTRODUCTION

The stigma is considered to be a glandular plant organ which provides a surface for pollen adhesion and germination preceeding fertilization. Numerous studies have been conducted on stigma exudates from many plants and, in general, were shown to be composed of glycolipids, carbohydrate and glycoproteins [1]. Little is known about the chemical composition of the stigma itself although a glycoprotein was identified in the styles of *Nicotiana alata* [2] and the stigmas of *Brassica oleracea* [3–5]. This glycoprotein has been implicated in self-incompatibility in plants during pollen–stigma interaction. During our studies on chemical and physiological barriers to cross-incompatibility in *Pennisetum americanum* stigmas to *Sorghum bicolor* and *Zea mays* pollen, it became evident that little was known about the amino acids present in the stigmas. In particular, we were interested in the amounts of amino acids present in the form of proteins and those which were in the free amino acid pool. The present study identifies the protein bound and free amino acids present in the stigmas of *Pennisetum americanum* (L.) Leeke from fertile and male-sterile plants at different developmental stages.

RESULTS AND DISCUSSION

The fertile and male-sterile whole stigmas were of comparable physiological age and were harvested at the youngest practical stage (I) i.e. just as the stigmas were emerging from the floret. Comparison of fertile and male-sterile stigmas could only be made at this stage of development since anther exsertion, pollen dehiscence and subsequent pollination of the stigmas occurred in the fertile plants at the later stages and the contribution of the pollen and pollen tubes could not accurately be assessed.

The total amino acids and *N* recovered from whole, non-extracted stigmas from fertile and male-sterile plants were similar and are shown in Table 1. Microkjeldahl *N* analyses of fertile and male-sterile stigmas were 4.7% and 4.9%, respectively, which indicated that *ca* 50% of the total *N* was in the form of free amino acids and/or proteins which were subsequently hydrolysed. The male-sterile stigma (I) hot water extract had more dry matter (DM) and total free amino acids extracted than the fertile stigma extract (Table 1). Again, the fertile and male-sterile (I) stigma extracts were prepared from stigmas that were of the same physiological and chronological age. As the male-sterile stigmas developed (stages II and III) the total free amino acid content and total *N* increased *ca* 3-fold. Although the DM extracted decreased 10 percentage units in stage II, this decrease was not reflected in the amino acid or *N* contents recovered (Table 1) and may be due to other water insoluble components such as polysaccharides.

The amino acid composition of whole, hydrolysed, unextracted fertile and male-sterile stigmas are in Table 2. Aspartic acid was the most predominant amino acid followed by glutamic acid, glycine and alanine. These four amino acids accounted for 45.8% and 50% of the total amino acids recovered from the fertile and male-sterile (I) whole stigmas, respectively. The remaining amino acids were present in significant but comparable amounts in both types of stigmas. The total amino acid contents, representing both free and protein bound acids, were comparable in fertile and male-sterile stigmas.

The soluble or free amino acids found in the hot water extracts of fertile and male-sterile stigmas at different developmental stages are in Table 2. Asparagine and glutamine predominated in the fertile and male-sterile (I) extracts. These two amino acids accounted for 58% of the total amino acids in both fertile and male-sterile (I) extracts. The aspartic and glutamic acid levels were comparable in both stigma extracts in contrast to their amidated counterparts. Of interest is the fact that the male-sterile (I) stigma extracts contained free proline while the fertile stigma extract did not.

Free asparagine levels increased during development in the male-sterile stigmas (Table 2). At stage III, asparagine

Table 1. Percentages of components present in whole stigmas and stigma extracts in different developmental stages (I, II, III) from male-sterile (MS) and fertile (F) *Pennisetum americanum* (L.) Leeke

	Whole stigmas			Stigma extracts		
			(%)			
Component	F	MS(I)	F	MS(I)	MS(II)	MS(III)
Dry matter extracted	0*	0*	24.6	40.6	30.6	40.0
Total amino acids recovered	16.8	16.7	9.2	10.1	22.4	27.9
Total *N* recovered as amino acids	2.3	2.2	1.0	1.1	2.5	3.3

*No extract was prepared since the whole stigmas were hydrolysed and analysed.

Table 2. Amino acid profiles* of whole stigmas and stigma extracts in different developmental stages (I, II, III) from male-sterile (MS) and fertile (F) *Pennisetum americanum* (L.) Leeke

Amino acid	Whole stigmas		Stigma extracts–Free amino acids			
	F	MS(I)	F	MS(I)	MS(II)	MS(III)
Lys	79.1	71.7	11.6	11.0	9.9	22.6
His	24.8	22.9	T§	T	12.3	32.0
Arg	58.1	51.7	T	T	17.6	56.8
Cys/2	1.1	1.6	T	T	T	T
Asp	213.8†	277.0†	99.0	95.7	96.1	46.5
Asn	—	—	269.1	354.6	987.6	1535.3
Thr	70.2	63.1	22.0	19.3	28.0	24.8
Ser	85.0	80.1	52.4	61.9	98.8	103.3
Glu	150.8‡	158.1‡	33.4	31.9	35.8	24.7
Gln	—	—	141.0	99.6	256.4	168.0
Pro	68.2	66.5	T	35.4	74.4	T
Gly	124.1	112.5	10.0	8.4	8.8	7.4
Ala	118.5	109.0	25.7	34.1	21.3	8.6
Val	79.2	73.5	20.3	16.0	23.1	17.2
Met	22.1	16.1	T	T	T	T
Iso	52.3	48.4	T	T	6.2	5.5
Leu	97.9	89.3	14.0	12.5	7.3	7.5
Tyr	35.7	32.9	T	T	T	T
Phe	44.9	41.7	T	T	9.1	18.9
Totals	1325.8	1316.1	698.5	780.4	1692.7	2079.1

*Results expressed as μ moles of amino acids/g dry wt. of tissue or extract.
†Value represents the total of asp plus asn because of hydrolysis.
‡Value represents the total of glu plus gln because of hydrolysis.
§Trace, < 1.0.

accounted for 74% of the free amino acids found in the male-sterile stigmas which would correspond to 20% of the DM contained in the extract. In addition, at stage II, free proline doubled in amount from stage I and only a trace was detectable at stage III. The significance of this is not known at this time. Overall, total free amino acid content increased *ca* 3-fold during development of the male-sterile stigmas which corresponded to the *N* and amino acid data in Table 1.

Asparagine is known to be a major nitrogen transporting compound in plants [6]. High concentrations of this amino acid and glutamine have been found in the xylem sap of peas which accounted for up to 70% of the xylem contents [7]. Asparaginase and asparagine aminotransferase are the two enzymes that normally metabolize asparagine [8]. Extracts of whole stigmas from fertile and male-sterile plants were prepared and exhibited very low activity for both of these enzymes. From these results, it is

obvious that asparagine is not metabolized and accumlates in the stigmas of male-sterile *Pennisetum*. The physiological or possible physical function of this amount of asparagine in the ontogeny of the sexual organs in *Pennisetum* is currently under investigation.

EXPERIMENTAL

Plant materials. Seeds of two lines of pearl millet, e.g. cytoplasmic male-sterile (Tift 23AE) *Pennisetum americanum* (L.) Leeke and male fertile Tift 23BE, were sown and grown in a greenhouse until they reached the boot stage of inflorescence development at which time they were moved to a plant growth room and grown under conditions described in ref. [9]. Stigmas were harvested from fertile and male-sterile plants just as they emerged from the floret (stage I) and from the male-sterile plants two days after emergence (stage II) and four days after emergence (stage III).

Extraction and analyses. Extracts were prepared by treating the stigmas (200–700 mg fr. wt) with 10 ml of boiling H_2O for 20 min. The suspension was cooled, filtered and the insoluble residue washed with 5 ml of hot H_2O. The filtrates were combined and lyophylized to dryness. Dry matter was determined by lyophilization because of small sample size, *N* according to ref. [10] and amino acid analysis by procedures in ref. [11]. Statistical analyses were not performed because of shortage of plant material.

Enzyme assays. Asparaginase (EC.3.5.1.1) was assayed according to ref. [12] with 20 mM K [13] included in the reaction mixture. Asparagine aminotransferase (EC.2.6.1.14) was determined by the method described in ref. [14].

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