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ANALYSIS OF THE FREE AMINO ACID CONTENT IN POLLEN OF NINE *ASTERACEAE* SPECIES OF KNOWN ALLERGENIC ACTIVITY

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Abstract: The study reports the free amino acid composition of the pollen of nine members of the family *Asteraceae*, i.e. *Ageratum conyzoides* L., *Blumea oxyodonta* DC., *Eupatorium odoratum* L., *Gnaphalium indicum* L., *Mikania scandens* Willd., *Parthenium hysterophorus* L., *Spilanthes acmella* Murr., *Vernonia cinerea* (L.) Lees. and *Xanthium strumarium* L. by thin layer chromatography. The amino acid content was found to vary from 0.5–4.0% of the total dry weight. Fourteen amino acids were identified, among which amino-n-butyric acid, aspartic acid and proline were present in almost all pollen samples. The other major amino acids present in free form included arginine, cystine, glutamic acid, glycine, isoleucine, leucine, methionine, ornithine, tryptophan and tyrosine.

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INTRODUCTION

Being the carrier of male genetic material, pollen is also essential for the life cycle of other living organisms, e.g. forming the principal source of normal non-liquid food for bees. Apart from this, the effect of airborne pollen on human health resulting in respiratory and skin disorders in sensitive patients has also been recognized. Hence, in recent years, a considerable amount of work has been carried out on various aspects of pollen biochemistry [1, 2, 3, 4, 6, 8, 9, 10, 11, 12, 13, 14, 15, 17, 18, 19].

Regarding the free amino acid composition of different pollen, all the essential amino acids have been reported to be present in pollen and total levels of free amino acids are usually higher in pollen than in leaves and other tissues [21]. At the same time, the concentration of all amino acids in pollen is considerably higher in bound form than in the free fraction. Stanley and Linskens [21] reported that the amino acid content can vary with climatic and nutritional conditions of the plants on which the pollen matures, as well as with storage and handling methods.

The present study reports the free amino acid composition of the pollen of nine members of the family *Asteraceae*, i.e. *Ageratum conyzoides* L., *Blumea oxyodonta* DC., *Eupatorium odoratum* L., *Gnaphalium indicum* L., *Mikania scandens* Willd., *Parthenium hysterophorus* L., *Spilanthes acmella* Murr., *Vernonia cinerea* (L.) Lees. and *Xanthium strumarium* L. This investigation was undertaken to study the free amino acid composition of the pollen and its homology between the various species of *Asteraceae*.

MATERIALS AND METHODS

The method of Sadasivam and Manickam [16] was used for the extraction as well as for the quantitative and qualitative analysis of the free amino acids. 500 mg of pollen sample was homogenized with 5–10 ml of 80% ethanol and centrifuged. The supernatant was preserved

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and the extraction was repeated twice with the residue. The pooled supernatants were collected and the volume reduced by evaporation. This extract was used for quantitative estimation of total free amino acids with ninhydrin reagent and the intensity of the purple colour developed was read using spectrophotometer at 570 nm, compared to pure reagent. A calibrated solution of glycine was used as standard.

Qualitative analysis of the free amino acids was carried out by thin layer chromatography (TLC) on DC-Alufolien Kieselgel 60 aluminium sheets (Merck) using n-butanol : acetic acid : water (80:20:20 v/v) as eluant. Then, 0.1% ninhydrin in acetone was used for the detection of amino acids by heating the sheets at 110°C for 5 minutes and the Rf values calculated. To quantify the amino acid content of each spot, the samples were chromatographed on two sheets under identical conditions. One sheet was sprayed with ninhydrin to identify the spots, and the positions corresponding to these spots were marked on the other sheet. Each spot from the unstained sheet was scraped off and the amino acid eluted with 5 ml of 80% ethanol and quantified according to the method of Sadasivam and Manickam [16] as mentioned earlier.

RESULTS

The free amino acid composition of the pollen of the nine investigated taxa is presented in Tables 1 and 2. The total amino acid content was found to be low in most of the taxa except in *Spilanthes acmella* where it was found to be 4%. In the other plants, the amino acid content ranged between 0.50–1.65%, thus proving that the amino acid content in pollen in the free fraction is considerably low.

Table 1. Free amino acid content of the pollen of investigated taxa.

Plant	Total free amino acid content (%)
Ageratum conyzoides	0.80
Blumea oxyodonta	0.65
Eupatorium odoratum	0.50
Gnaphalium indicum	0.85
Mikania scandens	0.80
Parthenium hysterophorus	1.40
Spilanthes acmella	4.00
Vernonia cinerea	0.85
Xanthium strumarium	1.65

TLC results revealed some degree of homology in the amino acid composition between the investigated taxa. Amino acids like amino-n-butyric acid, aspartic acid and proline were present in almost all the taxa. Proline was abundantly present in all the investigated samples constituting between 0.085 and 0.420 μmol/mg dry weight (Tab. 2). The other major amino acids present included glutamic acid, methionine, tryptophan and tyrosine. Histidine was present only in the pollen of

Table 2. Composition of free amino acids in the investigated pollen.

Amino acid	*A. conyzoides*		*B. oxyodonta*		*E. odoratum*		*G. indicum*		*M. scandens*		*P. hysterophorus*		*S. acmella*		*V. cinerea*		*X. strumarium*	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Amino-n-butyric acid	+	0.120	+	0.060	+	0.090	+	0.125	+	0.060	+	0.060	+	0.420	+	0.120	+	0.080
Arginine	-	-	-	-	-	-	+	0.130	-	-	-	-		-	-	-	+	0.090
Aspartic acid	+	0.070	+	0.090	-	-	T	0.010	+	0.040	T	0.020	T	0.010	T	0.010	+	0.090
Cystine	+	0.080	+	0.090	-	-	-	-	-	-	+	0.075	-	-	-	-	+	0.080
Glutamic acid	T	0.020	-	-	-	-	+	0.040	-	-	T	0.020	-	-	+	0.020	-	0.030
Glycine	T	0.030	-	-	-	-	-	-	-	-	-	-	T	0.009	-	-	-	-
Histidine	-	-	-	-	-	-	-	-	-	-	-	-	+	0.570	-	-	-	-
Iso-leucine	+	0.080	-	-	-	-	+	0.040	-	-	-	-	-	-	+	0.120	-	-
Leucine	-	-	-	-	-	-	+	0.075	-	-	-	-	-	-	+	0.060	-	-
Methionine	+	0.050	+	0.083	-	-	+	0.082	-	-	-	-	-	-	+	0.050	+	0.500
Ornithine	T	0.020	-	-	-	-	-	-	+	0.075	-	-	-	-	+	0.070	-	-
Proline	+	0.100	+	0.085	+	0.100	+	0.100	+	0.420	+	0.402	+	0.157	+	0.180	+	0.240
Tryptophan	+	0.090	+	0.071	-	-	-	-	-	-	+	0.092	-	-	-	-	+	0.200
Tyrosine	+	0.100	-	-	+	0.290	+	0.110	+	0.540	+	0.526	+	1.800	-	-	-	-
Unknown	-	-	+	0.046	-	-	+	0.060	+	0.320	+	0.081	+	0.910	+	0.095	+	0.100

A = presence; B = concentration in μmol/mg dry weight; + = present; T = present in trace amount; - = absent.

Spilanthes acmella while leucine was observed only in *Gnaphalium indicum* and *Vernonia cinerea.* Glycine was present only in trace amounts in *Ageratum conyzoides* and *Spilanthes acmella* and arginine was present in *Gnaphalium inducum* and *Xanthium strumarium.* Thus, a total of 14 amino acids were found to be present in free form in the members of *Asteraceae*. Apart from these, certain other amino acids were also present in the pollen samples of some species, which could not be identified from the standard amino acids and were categorised as unknown types.

DISCUSSION

Presence of amino-n-butyric acid, aspartic acid and proline in almost all the examined pollen samples reveals the nature of homology in the various members of *Asteraceae* which can be explained on the basis of their common ancestry, as has been earlier reported by Shellard and Jollife [21] who failed to detect any qualitative differences in the free amino acids extracted from pollen of 11 grass species. Increased levels of amino-n-butyric acid reflect the intensity of decarboxylation of the glutamic acid [21].

However, it is very difficult to draw any conclusion on evolution based upon the data on free amino acid content only, as amino acid composition greatly varies with climatic and nutritional conditions as well as with storage and handling patterns. Proline in the pollen is closely associated with their fertility and is involved in pollen tube formation and in other fundamental metabolic reactions associated with the sexual process [21]. Concentration of proline often increases under physiological stress and in such conditions other amino acids seem to be converted into proline to act as a reservoir of pollen amino acids [20].

Amino acids like arginine in certain pollen may have a role in storage and transport [7]. Kim *et al.* [5] reported that the levels of arginine, the amides (asparagine and glutamine) and proline increased significantly in pollen under increased nitrogen fertilisation. Glutamic acid, on the other hand, is a common substrate of glutamine, arginine and proline, and the primary NH_4^+ acceptor as well as a product of ammonia assimilation [7]. Thus, the accumulation of proline in all the examined pollen samples with the simultaneous absence of arginine in almost all the pollen could be reasoned as due to the competition for substrate of the enzymes in the arginine and proline biosynthesis, the accumulation of the products of which depends on a delicate balance of enzyme activity and substrate availability [22].

The amino acids which could not be identified in certain pollen samples may be among the various unusual amino acid-like compounds found in pollen. This is in conformity with the findings of Stanley and Linskens [21].

Although free amino acids in pollen are not directly involved in the allergenic reaction in human beings, they serve as precursors for proteins, which are main allergenic factors. The allergenicity of pollen depends upon their amino acid sequences that may markedly differ between genera and families.

CONCLUSION

The present findings indicate that amino-n-butyric acid, aspartic acid and proline constitute the major portion of free amino acids present in all the examined members of the family *Asteraceae*. This conforms with the results of other studies on the composition of amino acids in pollen.

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