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Full Length Research paper

ANALYSIS OF THE FREE AMINO ACID CONTENT IN POLLEN OF BANGLADESHI COMMON FRUITS FLOWER OF KNOWN ALLERGENIC ACTIVITY

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Abstract

The free amino acid content of aqueous extracts (preserved in 50 % glycerol) of pollen of Bangladeshi five (5) common fruits flower of the Asteraceae family three, i.e*. Mangifera indica* L, Pistacho and Cashew; Sapindaceae family one flower i.e. *Litchi chinensis* and Vitex glabrata family one flower i.e. Blackberry has been investigated using a thin-layer chromatographic technique. The amino acid content was found to vary from 0.75 to 2.25% of the total dry weight of pollen was estimated by a powerful oxidising agent (ninhydrin reagent). Thirteen amino acids were identified, among them n-butyric acid, proline and aspartic acid were present in almost all different pollen samples. The other major amino acids present in free from included alanine, cystine, arginine, glutamic acid, isoleucine, glycine, leucine, methonine, tryptophan and tyrosine. α-Alanine, γ-amino-*n*-butyric acid, glycine, dl-leucine, l-lysine, l-proline and dl-serine were identified. No species differences were detected and there was no difference between the viable and non-viable pollen extracts examined.

Key words: free amino acids, pollen, thin layer chromatography, fruits trees flower.

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 (1,2,3,4,5,6,7,8,9,10,11,12 and 13). Among the various aeroallergens, the role of pollen in causing respiratory disorders in sensitive patients has been greatly realized. Pollen allergy is caused by proteins, glycoproteins or even a single peptide which are present in the pollen wall and cytoplasm (2). The soluble proteins have been generally proved to be responsible for causing nasobronchial allergy. Thus the detection and characterization of allergy causing proteins or glycoproteins, or free amino acids is now a very challenging task for aerobiologists working in this field (14).

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 (15). At the same time, the concentration of all amino acids in pollen is considerably higher in bound from than in the free fraction. Stanley and Linskens (15) 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. It is also known that the abundance of particular taxa in a particular area could be responsible for the predominance of the pollen of those taxa in the atmosphere of that region. However in recent years, amino acid composition of seeds of *Capparis deciduas*; four improved lentil cultivars and leaves of *Capparis sepiaria* L; seeds of *Cicer arietinum* L. surveys carried out at various places in abroad (16, 17, 18 and 19). But amino acid composition studies of allergen flower pollen not yet sufficient.

The present study reports the free amino acid composition of the flower pollen of five members of three families' common & famous fruits trees in Bangladesh. This investigation was undertaken to study the free amino acid composition of the pollen and its homology between the various family and species of fruit trees.

MATERIALS AND METHODS

Source material

Pollen grains from the flower of the Asteraceae family, i.e*. Mangifera indica* L, Pistacho and Cashew; Sapindaceae family i.e. *Litchi chinensis* and Vitex glabrata family i.e. Blackberry were collected from the full bloomed flowers of the Nawabgang area Rajshahi, Bangladesh during the pollination period in March-April. Pollen from the anthers was sieved using different meshes (100, 200 and 300µm).To remove lipids and irritants of low molecular mass, the pollen sample was defatted with diethyl ether by repeated changes, until the ether become colourless. The defatted pollen powder was then completely vacuum-dried and store at 4^0C in airtight containers until further use.

Free amino acid extraction and Quantitative estimation:

The method of Sadasivam and Manickam (20) was used for the extraction as well as for the quantitative and qualitative analysis of the free amino acids. 500mg of defatted pollen sample was homogenized with 10ml of 80% ethanol and centrifuged at 10,000g for 20 minutes. The supernatant was preserved and the extraction was repeated twice (10ml+10ml) with the residue. The pooled supernatants were collected and evaporate it to dryness on a boiling water bath and dissolve the residue in 5ml of 0.2M citrate buffer (pH 5.0). Pipette 2ml of the above sample preparation in a test tube. For a reagent blank, take 2.0 ml of 0.2M citrate buffer (pH 5.0) in place of the sample preparation. In another set of tubes, take graded concentrations of glycine (0-100µg) and make the total volume to 2ml with 0.2M citrate buffer (pH 5.0). Add 1ml of KCN-acetone Ninhydrin reagent and mix thoroughly. Keep the test tubes in boiling water bath for 20 min, cool under running tap water and make the volume to 10ml with distilled water and the intensity of the purple colour developed was read using spectrophotometer (Spectronic-20, Japan) at 570nm, with reagent blank. Express the free amino acid content in flower pollen protein in terms of mg of glycine equivalents per gram of dry pollen.

Qualitative analysis of free amino acid by TLC:

Qualitative analysis of the free amino acids was carried out by the thin layer chromatography (TLC) on DC-Alufolien Kieselgel 60 aluminium sheet (Merck) using n-butanol:acetic acid:water (80:20:20 v/v) and chloroform:ethanol:acetic acid (20:10:3) as eluants. Then, 0.1% ninhydrin in acetone was used for the detection of amino acids by heating the sheets at 110^0C for 5 minutes and the R_f values calculated.

Calculation of R_f values

Measure the distance from the start line to the solvent front and to the front of each spot.

For each spot, calculate the R_f value (R_f means *relative to front*):

distance moved by spot
distance moved by solvent front

Compare the values you obtain with reference R_f values. Different solvents and different type's prepared TLC sheets (Lab prepared & Company prepared) will give slightly different results. One or both of the spots from solution X may be at the same level as another (known) amino acid alongside it. This should assist in identification.

Recovery of amino acids from TLC:

To quantify the free amino acid content of each above spots, 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 scared off and the amino acid eluted with 5ml of 80% ethanol and quantified according to the method of Sadasivam and Manickam (20) as mentioned earlier.

Table 1. Free amino acid content of the pollen of investigated different texa flower.

	Plant	Total free amino acid content (%) (gms/100gms flower with pollen)
1	*Litchi chinensis*	1.80%
2	*Mangifera indica L*	0.95%
3	*Blackberry*	0.75%
4	*Pistacho*	2.25%
5	*Cashew*	0.90%

RESULTS

The free amino acid composition of the pollen of the five investigated (three different families) famous fruits taxa in Bangladesh is presented in Tables 1 and 2. The total free amino acid content was estimated by a powerful oxidising agent and found to be low in most of the taxa pollen except *Pistaho flower pollen* where it was found around 2.25% and *Litchi chinensis* which was content 1.8%. In the other plants flower, the amino acid content ranged between 0.75 - 0.95%, thus proving that the amino acid content in pollen in the free fraction is considerably low.

Table-2. Composition of individual free amino acids in the investigated different flower pollen of fruit trees (µgm/mg of dry weight of pollen).

Amino acid	*Litchi chinensis*		*Mangifera indica L.*		*Pistacho*		*Cashew*		*Blackberry*	
	A	B	A	B	A	B	A	B	A	B
α-alanine	+	0.090	-	-	T	0.30	T	0.20	-	-
DL-leucine	+	0.30	+	0.08	+	0.32	-	-	T	0.06
L-lysine	T	0.025	T	0.02	_	_	_	_	_	_
L-proline	+	0.220	+	0.35	+	0.95	+	0.75	+	0.100
DL-serine	-	-	-	-	-	-	T	0.02	T	0.012
Tryptophan	+	0.095	_	_	T	0.025	T	0.12	_	_
Tyrosine	-	-	T	0.08	T	0.02	-	-	-	-
n-butyric acid	+	0.210	+	0.60	+	0.120	+	0.80	+	0.55
Aspartic acid	+	0.85	+	0.98	+	0.55	+	0.85	+	0.45
arginine	+	0.55	+	0.65	T	0.06	T	0.08	+	0.43
Isoleucine	T	0.080	-	-	T	0.11	-	-	T	0.04
Glutamic acid	T	0.03	_	_	T	0.02	_	_	_	_
Methonine	+	0.09	_	_	T	0.02	+	0.65	+	0.110
Unknown	+	0.210	+	0.89			+	0.65		

A= Presence; B= Concentration in µgm/mg dry weight; += Present; T= Present in trace amount; - = absent.

Table-3. R_f values of different free amino acid present in individual flower pollen by two different solvent systems.

Amino acid	*Litchi chinensis*		*Mangifera indica L.*	*Pistacho*		*Cashew*		*Blackberry*	
	A	B		A	B	A	B	A	B

			A	B						
α-alanine	0.36	0.35	0.41	0.37	0.38	0.39	0.34	0.33	0.41	0.43
DL-leucine	0.73	0.71	0.68	0.71	0.67	0.73	-	-	0.69	0.66
L-lysine	0.13	0.12	0.14	0.12	_	_	_	_	_	_
L-proline	0.44	0.41	0.46	0.42	0.41	0.40	0.43	0.39	0.39	0.37
DL-serine	0.27	-	-		0.26	-	-	-	0.28	-
Tryptophan	0.67	0.65	_	_	0.62	0.65	0.60	0.55	_	_
Tyrosine	-	-	-	-	-	-	-	-	-	-
n-butyric acid	0.29	0.26	0.40	0.60	0.32	0.33	0.29	0.31	0.34	0.35
Aspartic acid	0.23	0.25	0.26	0.28	0.22	0.25	0.24	0.25	0.21	0.24
arginine	0.19	0.21	-	-	0.24	0.29			0.21	0.25
Isoleucine	0.71	0.69	-	-	-	-	-	-	0.68	0.64
Glutamic acid	0.30	0.33	_	__	0.29	0.28	_	_	_	_
Methonine	0.55	0.53	_	_	0.52	0.56	0.50	0.55	0.56	0.51
Unknown	0.09	0.14	0.31		0.71	0.43			0.49	0.49

A= n-butanol:acetic acid:water (80:20:20) and B= butanol:acetic acid:water (12:3:5)

TLC results with two different solvent systems [n-butanol:acetic acid:water (80:20:20 v/v) and chloroform:ethanol:acetic acid (20:10:3)v/v] revealed some degree of homology in the amino acid composition between the investigated fruits taxa flower pollen. Maximum numbers of R_f values of unknown and standard amino acids were overlapping and that gives us the clear idea about unknown amino acid identification. Amino acids like n-butyric acid, aspartic acid, proline, and arginine were present in almost all the taxa pollen. n-butyric acid and proline were abundantly present in all the investigated samples constituting between 0.35 and 220µgm/mg dry weight (Tab. 2). The other major amino acids present included metionine, proline, leucine, alanine. Lysine was present trace amount only in the pollen of *Litchi chinensis* and *Mangifera indica L.* while serine was observed only in *Cashew and Blackberry.* Tyrosine was present only in trace amount in *Mangifera indica and Pistacho.* Isoleucine and glutamic acid were present in trace amount *Litchi chinensis and Pistacho.* Thus, a total of 13 amino acids were found in free from in the Pollen grains from the flower of the Asteraceae family, i.e. *Mangifera indica* L, Pistacho and Cashew; Sapindaceae family i.e. *Litchi chinensis* and Vitex glabrata family i.e. Blackberry. 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.

DISCUSSION

Presence of n-butyric acid, aspartic acid, proline, and arginine in almost all the examined pollen samples reveals the nature of homology in the various members of common fruirs pollen examined on the basis of their common ancestry, as has been explained earlier reported by Stanley *et al*, (14). Those researcher extracted free amino acids from pollen of 11 grass species and concluded the increased level of n-butyric acid reflect the intensity of decarboxylation of the glutamic acid (14).

Amino acids like arginine in certain pollen may have a role in storage and transport (21). Kim *et al*. (22) reported that the levels of arginine, the amides (asparagines 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+ acceptor as well as a product of ammonia assimilation (21). Thus, the accumulation of proline in all most 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 (23).

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 (14). 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 (24).

The amino acids which could not be identified in certain pollen samples may be among the various unusual amino acids-like found in pollen. This is in conformity with the findings of Stanley and Linskens (14). 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 allergenic of pollen depends upon their amino acid sequences that may markedly differ between genera and families, which reported earlier by Mondal, *et al*., (25).

CONCLUSION

The present findings indicates that n-butyric acid, aspartic acid, proline, and arginine constitute the major portion of free amino acids present in all the examined members of Bangladeshi common fruits pollen with different families. This confirms with the results of other studies on the composition of amino acids in pollen.

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