RESEARCH ARTICLE

Analysis of free amino acids and total protein content in some allergenic pollen grains in Nagpur (MS) India

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ABSTRACT

Pollen grains contain number of metabolites which are responsible for physiological and metabolical activities. Pollen grains release the proteins, free amino acids and other chemical metabolites at different rates and quantities. The differences in these metabolites may be responsible for the control rate and type of allergic reactions caused by pollen grains. *Albizia lebbeck, Ipomoea fistulosa, Ricinus communis, Moringa oleifera, Prosopis juliflora* are some known allergenic pollen producing plants from Nagpur region. The present paper deals with analysis of free amino acids and total proteins content in some of these plants pollen grains . The biochemical evaluation of these metabolites was performed by standard methods. The free amino acids were separated and identified by thin layer chromatography and estimation of protein was done by Lowry's method. Among all the species the highest number of amino acids were found in *Albizia lebbeck* (17), followed by *Prosopis juliflora* (16), *Ipomoea fistulosa* (16), *Moringa oleifera* (15) and *Ricinus communis* (14) while highest protein content was found in *Albizia lebbeck* (13.27%) followed by *Prosopis juliflora* (11.46%), *Ipomoea fistulosa* (10.64%), *Moringa oleifera* (9.57%), *Ricinus communis*(8.56%).

Key words: Allergenic pollen, amino acids, protein.

INTRODUCTION

Human allergic diseases can be caused by many substances in the environment. Pollen grains are the major constituent of the organic micro – bodies in the atmosphere, which cause allergic diseases like hay fever, asthma and dermatitis (Basarkar and Saoji 2006).

Pollen grains contains the number of metabolites like protein, nucleic acids, carbohydrates, lipids and vitamins which are responsible for physiological and metabolical activities (Stanley et al., 1974). They release the proteins and other chemical metabolites to the environment at different rates and in different quantities which depend primarily on specific differences such differences are responsible for the control rate and type of allergenic reactions caused by different types of pollen.

Pollen from plants like grasses, Parthenium, Hysterophorus, Amarantaceae, Chenopodiaceae complex, Eucalyptus terreticornis, Equesetifolia, Cassia siamia etc. are found allergenic as per clinical data at Nagpur (Kalkar and Patil, 1994 ; Kalkar *et al.,*1998).

Considerable work has been carried out by various workers on chemistry and biochemistry of allergenic pollen grains of various plant species (Chakraborty et al.(1996), Mondal *et al.*(1998), Parai *et al*. (1998), Boral *et al.*(1999), Parui *et al*. (1999) and Parui *et al.*(2002). Hence the chemical nature of pollen grains of *Albizia lebbeck, Ipomoea fistulosa, Ricinus communis, Moringa olifera, Prosopis juliflora* which is supposed to be allergenic, has been investigated.

MATERIAL AND METHODS

The extraction as well as qualitative analysis of the free amino acids and estimation of protein was performed by standard methods (Sadasivam and Manickam,1992 ; Lowry *et al.,* 1951).100mg of pollen powder sample was homogenized with 10 ml of 70% alcohol for half an hour. Alcoholic extract was separated by centrifugation at 6000-7000 rpm and evaporation. This extract was used to perform various qualitative tests and aliquot portion of the same were used for TLC study for the analysis of free amino acid.
Qualitative analysis of the free amino acids by thin layer chromatography (TLC) was carried out on square silica gel coated glass plate of 20cm+20cm, using n-butanol, acetic acid and water in ratio of 4:1:5 (v/v) respectively as eluent. Then 0.2% ninhydrin in butanol was used for detection of amino acids by heating the plates at 100-110^{o}c for 5 to 10 minutes and the Rf values were calculated.

Extraction of protein from pollen was carried out with trichloacetic acid, then with this extract various qualitative and quantative estimation of potein were performed by standard methods (Buzarbarua ,2000 ; Lowry *et al*., 1951).

RESULTS AND DISCUSSION

The free amino acids of the pollen grain were studied by different method (Table 1 and 2). The qualitative test like Ninhydrin test, Million's reaction, Xanthoproteic test have shown the positive results confirmed the presence of amino acids,proline, tyrosine, tryptophan and arginene (Table 3).

The TLC of pollen extract of all investigated plants along with samples of authentic amino acids in different pollen grains of studied plants.The common free amino acids like alanine, 2aminobutyrine, arginine, glycine, leucine, lysine, aspartic acid, ornithine, methionine, phenylalanine, tryptophan, tyrosine, proline were found in all the studied taxa. Although free amino acids in pollen are not directly involved in the allergenic reactions in human beings, they serve as precursors for proteins, which are main allergenic factors. The allergy of pollen depends upon their amino acid sequences that may markedly differ between genera and families, which has reported earlier by Mondal, *et al.* (Mondal *et al.,* 1998).

The amount of protein present in the pollen of five investigated taxa. The highest amount of protein was found in *Albizia lebbeck* (13.27% followed by *Prosopis juliflora* (11.46%), *Ipomoea fistulosa* (10.64%), *Moringa olifera* (9.57%) and *Ricinus communis* (8.56%)(Table 3).

Table1: Qualitative analysis of free amino acids and protein by preliminary biochemical screening of pollen grains of studied plants

Test	*M.oleifera*	*P.juliflora*	*A.lebbeck*	*R.communis*	*I. fistulosa*
A)Test for Amino acids					
1)Ninhydrin test	+	+	+	+	+
2)Xanthoproteic test	+	+	+	+	+
3)Glyoxylic test	-	+	+	-	+
4)Pauly test	-	-	-	-	+
5)Million's test	+	+	+	+	+
B)Test for proteins					
1)Biurette test	-	-	+	+	+
2)Xanthoproteic test	+	+	+	+	+
3)Glyoxylic test	-	+	+	-	+
4)Formaldehyde test	-	-	+	-	+

Table 2.Analysis of free amino acids by thin layer chromatography of studied plants.

Band Number	Sample	*M.oleifera*	*P.juliflora*	*A.lebbeck*	*R.communis*	*I.fistuiosa*
1	DL-Alanine	+	+	+	+	+
2	DL-2Aminobutyrine	+	+	+	+	+
3	L-Arginine	+	+	+	+	+
4	DL-Threonine	-	-	-	-	-
5	L-Cysteine Hydrochloride	-	-	-	-	-
6	L-Cysteine	-	-	-	-	-
7	L-Glutamic acid	-	+	+	+	+
8	Glycine	+	+	+	+	+
9	L-Histidine	-	-	-	-	+
10	DL-nor-Leucine	+	+	+	+	+
11	DL-iso-Leucine	+	+	+	+	+
12	L-Leucine	+	+	+	-	-
13	L-Lysine	+	+	+	+	+
14	DL-Aspartic acid	+	+	+	+	+
15	L-Ornithine	+	+	+	+	+
16	DL-Methionine	+	+	+	+	+
17	DL-B-Phenylalanine	+	+	+	+	+
18	DL-Serine	-		+	-	-
19	DL-Tryptophan	+	+	+	-	+
20	L-Trosine	+	+	+	+	+
21	DL-Valine	-	-	-	-	-
22	L-Proline	+	+	+	+	+
23	3(3,4-Dihydroxy Phenyl	-	-	-	-	-
Total Amino acids		15	16	17	14	16

Where, - = Absent and + = Present

Table 3: Quantitative estimation of protein by Lowery Method of studied plants

S. No.	Name of plants	Percentageof proteins	Average(%)
1	*M.oleifera*	9.58	9.57
		9.55	
		9.59	
2	*P.juliflora*	11.47	11.46
		11.48	
		11.44	
3	*A.lebbeck*	13.29	13.27
		13.25	
		13.28	
4	*R.communis*	8.55	8.56
		8.58	
		8.57	
5	*I. fistulosa*	10.63	10.64
		10.65	
		10.64	

In present investigation for analysis of amino acids, proline was found in pollen of all investigated taxa. The potential role of proline in pollen tube growth has also been suggested (Dashek *et al* .,1970). Amino acids like arginine in certain pollen may have a role in storage and transport (Miflin *et al*., 1997 ; Kim *et al.,*1987). It is very difficult to draw any conclusion on evolution based upon the data on free amino acid content only, as free amino acid composition greatly varies with climatic and nutritional condition greatly varies with climatic and nutritional conditions as well as with storage and handling patterns (Iwanami

,1959). Proline in 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 (Stanley and Linksens 1974). Prolin have also reported in role of protein synthesis (Zhang *et al*., 1982).

CONCLUSION

The biochemical investigations in pollen grain of *Ipomoea, Prosopis, Ricinus, Moringa, Albizia* pertaining to amino acids and protein synthesis were studied. The differences in amount of biochemical content may cause allergy, differing from region to region. Variation in amount of amino acids an proteins, may be the reason for the allergenicity of *Ipomoea, Prosopis, Ricinus, Moringa, Albizia.*

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