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Amino acids composition of pollen grains of some medicinally important plant species

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ABSTRACT

Natural plant products as nutrition supplements have gained lots of attention these days. Among different plants products, apicultural products such as pollen grains are widely used as medicines and dietary supplements due to presence of essential nutrients like amino acids, proteins, flavonoids and alkaloids. As the composition of different essential and non-essential amino acids defines the accurate nutritional value, the present study focuses on composition of free amino acids in pollen grains of some medicinally important plant species viz., Bauhinia purpurea, Bauhinia variegata, Cassia biflora, Cassia fistula, Cassia glauca, Cassia saimea, Delonix regia, Hibiscus rosa sinensis and Melia azadirach. 18 different amino acids were analyzed using amino acid analyzer. Among all plant species, it was observed that Cassia fistula had maximum number (17) of amino acids. The order of different plant species on the basis of number of amino acids was Cassia fistula (17) > Melia azadirach (16) > Bauhinia purpurea (13) = Cassia biflora (13) = Hibiscus rosa sinensis (13) > Bauhinia variegata (12) > Cassia glauca (11) = Delonix regia (11) > Cassia saimea (10). It was found that four amino acids viz., aspartic acid, asparaginine, β-Alanine and alanine were present in all plant species studied.

Keywords: Apiculture, *Cassia fistula*, Nutrition, Natural diet supplements.

INTRODUCTION

From the beginning of human civilization, when natural plant parts or plants as whole were used as home remedies to cure ailments, to the present world of synthetic medicines, plants remained an integral part of medicinal world [1]. As the use of commercial medicines can develop multiple drug resistance in human beings, researchers are looking for herbal medicines which can cure diseases and do not possess any ill side effects on human health. Accordingly, development of natural novel drugs from different parts of plants *viz.*, leaves, stems, roots, fruits, seeds, pollens etc. have been the main area of research in pharmaceuticals [2-3]. Phytochemical analysis of pollen grains of various plants species showed presence of alkaloids, phenolic compounds, flavonoids, saponins, steroids, tannins, terpenes, proteins, amino acids, fatty acids etc [4-5]. Due to presence of these bioactive compounds pollen grains have been shown to posseses many bioactivities like antifungal, antibacterial, antioxidant, antimutagenicity, anti-inflammatory etc. [6].

In the present study, pollen grains of nine plant species viz., *Bauhinia variegata, Bauhinia purpurea, Cassia biflora, Cassia fistula, Cassia glauca, Cassia saimea, Delonix regia, Hibiscus rosa sinensis and Melia azadirach* were studied for free amino acid composition. Although various kinds of bioactivities of different parts of these plant species have been well documented but no report is available for use of their pollen grains. For example, leaves of *Bauhinia variegata* have antibacterial, anti-inflammatory, antitumor and antiobesity activities [7-8]. *Bauhinia purpurea* possesses antifungal, anti-inflammatory, antimalarial, antibacterial properties [9]. Veerachari and Bopaiah [10] reported some active phytoconsituents in the leaves of *Cassia biflora*. Seeds of *Cassia fistula* possess antimicrobial activity [11] while leaves of *Cassia fistula* possess antioxidant and cytotoxic activities [3]. Bioactivities such as antioxidant, antibacterial and antidiabetic of *Cassia glauca* were reported by Salahiddin and

Jalalpura [12]. Leaves of *Cassia saimea* have shown antibacterial, anti-inflammatory, antimicrobial and cytotoxic activities [13-14]. Mariajancyrani *et al*. [1] evaluated the antioxidant potential of *Delonix regia.* Kumar and Singh [15] reported that *Hibiscus rosa sinensis* was used to cure many diseases like diarrhea, fever, bronchial catarrh, skin problems. Antibacterial activities of *Melia azadirach* have been studied by Ramya *et al*. [16].

Pollen grain is a male gametophyte in the process of fertilization in all angiosperms and gymnosperms and is enriched with all nutrients which are required for plant growth and development. It is a good natural food because of having high nutrition value [4, 17]. Pollen contains all essential and nonessential amino acids which are building blocks of proteins. However, the concentration of amino acids varies from species to species and depends on factors like abiotic and biotic stress, climatic condition and nutrition conditions of plants [17]. Out of all 24 amino acids, 10 amino acids are essential *i.e*. they cannot be created from other compounds by human body and are taken as food. So the study of natural plant material is very important which are enriched with all essential amino acids. Composition of amino acids defines more accurate nutritional value of pollen as compared to concentration of protein [4,18-19]. Keeping this in mind, the present study was planned to estimate the composition of free amino acids in pollen grains of nine medicinally important plant species.

EXPERIMENTAL SECTION

1.1. Plant species

The pollen grains of nine plant species were collected and analyzed for composition of amino acids. The collected pollen grain samples were coded as described in Table 1.

Table 1. List of plant species and their sample codes

S. No.	Plant Name	Family	Sample code
1.	*Bauhinia variegata* L.	Fabaceae	BV
2.	*Bauhinia purpurea* L.	Fabaceae	BP
3.	*Cassia biflora* L.	Fabaceae	CB
4.	*Cassia fistula* L.	Fabaceae	CF
5.	*Cassia glauca* (Lam.)	Fabaceae	CG
6.	*Cassia saimea* (Lam.)	Fabaceae	CS
7.	*Delonix regia* (Boj. ex Hook.) Raf.	Fabaceae	DR
8.	*Hibiscus rosa sinensis* L.	Malvaceae	HR
9.	*Melia azadirach* L.	Meliaceae	MA

1.2. Collection of pollen grains

Fresh flowers (about to anthesis) of all plant species were collected from Guru Nanak Dev University campus, Amritsar, Punjab (India). Anthers were isolated from the flowers and the pollen grains were collected in pre weighted Petri plates by teasing the anthers with the help of sharp forceps and tapping in Petri plates. The weight of Petri plates with pollen was noted again. 1 g of pollen grains was obtained from approximately 100-150 flowers.

1.3. Preparation of pollen extracts

Pollen extracts were prepared by following the protocol given by Carpes *et al*. [6] with some modifications. 1 g of collected pollen grains were extracted with 70 % ethanol (7.5 ml) by 1 min shaking at interval of 10 min at 70ºC temperature for one hour. The supernatant was separated and the solid residue left behind was re-extracted with 70 % ethanol for 3-4 times. The collected supernatant was considered as pollen extract and stored at 4 ºC till further analysis.

1.4. Analysis of amino acids

For amino acids analysis, 4 ml of ethanolic extract was hydrolyzed at 110º C for 16 h in 2 ml of 6N HCl. The solid residue left behind were dissolved in 0.01 N HCl and filtered through 0.2 µm filter before use. Different amino acids were analyzed using amino acid analyzer (Shimadzu, Model No. NEXERA X2).

1.5. Statistical analysis

Pearson correlation was used to check the correlation between amino acids of various plant species.

RESULTS AND DISCUSSION

The content and number of amino acids present in the pollen grains of plants under study is given in Table 2. The order of different plant species on the basis of maximum number of amino acids analyzed in their pollen grains was observed as *Cassia fistula* (17) > *Melia azadirach* (16) > *Bauhinia purpurea* (13) = *Cassia biflora* (13) = *Hibiscus rosa sinensis* (13) > *Bauhinia variegata* (12) > *Cassia glauca* (11) = *Delonix regia* (11) > *Cassia saimea* (10).

Among the total amino acids studied, aspartic acid, asparaginine, β-alanine and alanine were present in pollen grains of all species studied whereas γ-amino butyric acid, histidine, cysteine, ornithine and lysine present in eight of nine species studied. Phenylalanine was observed only in one species (*Cassia glauca*).

Pollen grains samples showed presence of various essential and non-essential amino acids [4, 17-18]. In the present study, various essential amino acids *viz.*, arginine, histidine, leucine,

Table 2. Contents of different amino acids (µg/L) identified in pollen grains of various plant species

Amino acid	Plant species								
	BP	BV	CB	CF	CG	CS	DR	HR	MA
Essential amino acids									
Arginine	71.302	132.377	-	33.438	797.715	46.993	-	44.954	42.709
Histidine	627.1	1646.367	1515.081	1029.562	-	403.285	684.697	264.205	1221.778
Leucine	273.855	567.151	414.481	537.2	45.834	107.903	-	-	389.119
Lysine	1894.141		2453.416	2776.789	1572.231	103.995	894.046	1629.198	1385.598
Phenylalanine	-	-	-	-	29.969	-	-	-	-
Threonine	-	-	-	41.508	-	-	89.766	-	-
Valine	-	3845.009	1715.708	2670.329	-	-	1458.743	159.765	1869.478
Non essential amino acids									
Alanine	930.044	3750.314	1515.192	3065.195	89.622	224.414	899.096	82.409	1792.17
Aspartic acid	943.287	1386.895	4050.856	1330.043	299.278	392.3	2353.218	162.878	4857.677
Asparaginine	366.596	400.877	496.328	229.155	27.124	160.664	298.057	79.472	442.441
B-Alanine	304.852	545.047	746.376	2921.237	320.929	92.999	2533.119	55.509	635.084
Cystine	625.003	1390.035	867.584	1552.776	101.898	-	320.799	36.662	835.054
γ- amino butyric acid	341.002	2382.658	439.496	3259.255	-	108.313	368.017	171.538	181.225
Glutamine	-	-	-	243.063	-	-	-	-	172.88
Glycine	776.302	-	-	2782.015	-	-	-	-	725.434
Proline	-	14.442	17.352	17.301	-	-	-	43.096	12.572
Ornithine	406.339	-	503.207	599.431	177.334	144.384	196.288	56.622	483.457
Tyrosine	175.364	60.666	328.933	570.588	102.655	-	-	34.856	176.509

BP: *Bauhinia purpurea;* BV: *Bauhinia variegata;* CB: *Cassia biflora;* CF: *Cassia fistula*; CG: *Cassia glauca;* CS: *Cassia saimea;* DR: *Delonix regia:* HR: *Hibiscus rosa sinesis*; MA: *Melia azadirach.*

lysine, phenylalanine, threonine and valine were found in the pollen grains of different plant species which indicated the nutritional importance of the pollen grains. The presence of free essential and non-essential amino acids is considered to be more important to indicate the nutrition value of pollen than the contents of proteins [4]. Pollen grains of different plant species have shown many types of bioactivities [4, 6, 20]. An anti-inflammatory and antioxidant activity of pollen grains of pine was reported by Lee *et al*. [21]. Pascoal *et al*. [20] studied the antimicrobial, antimutagenic, antioxidant and anti-inflammatory activity of bee pollen. Pinto *et al*. [22] evaluated the antigenotoxicity of bee pollen using micronuclei assay in human lymphocytes. Polysaccharides derived from pollen grains of *Brassica napus* L. have shown antitumor activity in Sarcoma 180-bearing mice and B16 melonoma-bearing mice [23]. Pollen grains of olive and palm possess antioxidant and antimicrobial activities [4]. Barzin *et al*. [24] reported the antimutagencity of pollen grains of *Phoenix dactylifera* using Ames assay. These bioactivities of pollen grains are due presence of active phytoconstituents [20, 25].

Pearson correlation was used in the present study to find out the correlation between different amino acids present in pollen grains of various plant species (Table 3). Correlation analysis showed that amino acid arginine (ARG) had positive correlation with phenylalanine (PHE) 0.01 % level of significance. Arginine is important for the detoxification and is effective in relieving muscular cramps while phenylalanine is important for reduction of depression. Histidine (HIS) showed positive correlation with valine (VAL), alanine (ALA), aspartic acid (ASP), asparagine (ASN), cysteine (CYS). Histidine (HIS) regulates and utilizes essential trace elements like iron, copper, zinc, molybdenum and manganese [26] whereas aspartic acid (ASP) plays role in regulating energy cycle and acts as an important element to remove toxic elements from the cell which can damage brain and nervous system [27]. Alanine protects the cells from damages during intense physical activity by converting simple sugar to energy [28]. Cysteine (CYS) is important for proper functioning of skin and formation of skin cells. It is also important for recovery of nail and hair tissue [29]. Amino acid leucine (LEU) had significant correlation with amino acids such as valine (VAL), alanine (ALA), asparagine (ASN), cysteine (CYS) and γ-amino butyric acid (GABA). It was earlier reported that asparagine (ASN) and leucine (LEU) play an important role in biosynthesis of glycoproteins, regulate the synthesis of bile salts, act as inhibitory neurotransmitters, promote growth and recovery of new muscles while amino acid γ-amino butyric acid (GABA) is a chief inhibitory neurotransmitter in mammalian central nervous system [30-31]. Amino acid lysine (LYS) showed positive correlation with ornithine (ORN) and tyrosine (TYR). Ornithine plays major role in urea cycle and has antifatigue effects. Lysine (LYS) regulates the carbohydrate metabolism and help in production of energy.

Table 3. Correlation matrix to show relationship between different amino acids identified in pollen grains of various plant species

	ARG	HIS	LEU	LYS	PHE	THR	VAL	ALA	ASP	ASN	BALA	CYS	GABA	GLN	GLY	PRO	ORN	TYR
ARG	1																	
HIS	-0.503	1																
LEU	-0.286	0.855**	1															
LYS	-0.016	0.023	0.202	1														
PHE	0.987**	-0.542	-0.351	0.063	1													
THR	-0.246	-0.025	-0.205	0.042	-0.174	1												
VAL	-0.246	0.857**	0.799**	-0.073	-0.353	0.204	1											
ALA	-0.299	0.836**	0.909**	0.013	-0.366	0.084	0.952	1										
ASP	-0.375	0.674*	0.419	0.214	-0.323	0.085	0.420	0.316	1									
ASN	-0.558	0.875**	0.660*	0.068	-0.571	-0.005	0.563	0.563	0.796**	1								
BALA	-0.268	0.226	0.220	0.340	-0.206	0.862	0.464	0.421	0.198	0.120	1							
CYS	-0.295	0.816**	0.948**	0.273	-0.348	0.066	0.873**	0.960**	0.373	0.595	0.473	1						
GABA	-0.199	0.519	0.738**	0.157	-0.257	0.212	0.779**	0.866**	-0.072	0.162	0.577	0.857**	1					
GLN	-0.203	0.282	0.527	0.460	-0.185	0.171	0.417	0.494	0.344	0.135	0.558	0.597	0.558	1				
GLY	-0.201	0.171	0.520	0.590	-0.193	0.228	0.314	0.481	0.035	0.043	0.629*	0.630*	0.694*	0.874**	1			
PRO	-0.301	0.133	0.092	0.281	-0.308	-0.227	0.162	0.106	-0.011	-0.094	-0.109	0.113	0.178	0.145	0.070	1		
ORN	-0.258	0.330	0.475	0.776**	-0.187	0.093	0.134	0.243	0.565	0.449	0.437	0.476	0.212	0.685	0.696*	-0.092	1	
TYR	-0.152	0.389	0.654*	0.804**	-0.117	0.054	0.373	0.500	0.276	0.258	0.525	0.701	0.617	0.734	0.843**	0.156	0.841**	1

- **Data is significant at $P \leq 0.05$.*
- *** Data is significant at $P \leq 0.01$*

ARG: arginine; HIS: histidine; LEU: leucine; LYS: lysine; PHE: phenylalanine; THR: threonine; VAL: valine; ALA: alanine; ASP: aspartic acid; ASN: asparagine; BALA: B-alanine; CYS: cysteine; GABA: γ- amino butyric acid; GLN: glutamine; GLY: glycine; PRO: proline; ORN: ornithine; TYR: tyrosine.

It also acts as precursor of synthesis of amino acid carnitine which helps in transportation of long chain fatty acids into the mitochondria for energy production [32]. Valine (VAL) and alanine (ALA) showed positive correlation with γ- amino butyric acid (GABA), cysteine (CYS) while amino acid glutamine (GLN), β-alanine (BALA) and γ- amino butyric acid (GABA) have positive correlation with glycine (GLY). Glycine acts as inhibitory neurotransmitter in central nervous system [33]. The composition of free amino acids has been reported to be higher in pollen grains as compared to leaves and other tissues. Although contents of amino acids vary from plant to plant, yet the nutritional status of plant also depends upon environmental conditions [17].

CONCLUSION

Pollen grains contain many kinds of nutrients including various free essential and non-essential amino acids which are necessary for human body. In the present study, 7 essential amino acids viz., arginine, histidine, leucine, lysine, phenylalanine, threonine and valine and 11 non-essential amino acids viz., alanine, aspartic acid, asparagine, β-alanine, cysteine, GABA, glutamine, glycine, proline ornithine and tyrosine were observed in pollen grains of different plant species confirming the nutritional value of pollen grains. The presence of essential and non-essential amino acids not only enhances the nutritional value but also has great role in enhancing the medicinal properties of particular plant species. Among different plant species studied, the pollen grains of *Cassia fistula* plant showed presence of maximum number of amino acids followed by *Melia azadirach, Bauhinia purpurea, Cassia biflora, Hibiscus rosa sinensis, Bauhinia variegata, Cassia glauca, Delonix regia* and *Cassia saimea.*

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