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PRE AND POST POLLINATION CHANGES IN AMINO ACIDS AND MINERAL COMPOSITIONS OF ANTHER AND STIGMA IN *SOLANUM SURATTENSE BURM.F*

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ABSTRACT

In the present study attempts are made to understand the role of free amino acids and minerals present in pollen and pistil before, during and after pollination stages in *Solanum surattense* by using the techniques of paper chromatography and atomic absorption spectrometry respectively. It is found that in the anther 15 types of amino acids out of 20 types are present during and after pollination stages. But before pollination only 11 types of amino acids were noticed in the anther. This indicates that during pollination four new amino acids viz. arginine, aspartic acid, ornithine and valine are synthesized. Similarly, stigma also contained 16 types of amino acids during and after pollination and only 11 types of amino acids before pollination indicating synthesis of 5 new types of amino acids viz. aspartic acid, lysine, ornithine , proline and valine during pollination. Three major elements i.e. K, Ca, and Mg and nine minor elements i.e. Na, Cu, Mn, Ni, Zn, Pb, Fe, Cd and Cr were detected in the stigma before, during and after pollination stages. A considerable decrease in the amount of K and Mg but increase in Ca was reported from before to after pollination stages. Among the minor elements, Ni, Zn and Fe showed considerable decrease, while Cu, Mn, Pb, Cd and Cr showed increase in their amount. The present paper describes the role of amino acids, and major and minor mineral elements in pollen –pistil interaction.

Key words: *Amino acids, pollen, germination, major elements, minor elements, Solanum surattense.*

INTRODUCTION

Pollen biology is not only the oldest branch of plant physiology, but pollen itself has also become the area of remarkable interest for interdisciplinary research. Numerous studies on pollen biology including palynology have been carried out to understand reproductive success which includes SEM studies of pollen and stigmatic surface [1], [2]; pollen viability [3]; *in vitro* pollen germination [4]; effects of light, hormones, pH, pesticides, herbicides etc. on pollen germination [5],[6]; pollen pistil interaction [7], [8] and role of amino acids in pollen germination and tube growth [9], [10], [11], [12]. However, the precise relationship between the various amino acids and the physiological status of pollen and stigma is not very clear.

Pollen often requires minor and major elements for germination and elongation of tube. An abundance of calcium signal receptivity in the ovary and ovule provides essential mineral nutrition and guides the pollen tube in some plants [13]. Along with the major elements found in plant tissue, pollen also contains many minerals in trace and micro quantities. Most essential elements with the exception of boron and possibly calcium are present in mature pollen at levels sufficient to facilitate normal growth and fertilization. Potassium, phosphorous and iron are the most commonly occurring minerals in pollen. The influence of heavy metals[14]; calcium [13]; zinc [15] , [16] accumulated S, Mn, Al, Na, Cu, Ni, Zn and Cd [17] and Co, Mn, and Zn [18] were studied during pollen germination and tube growth.

In the present work attempts are made to understand the role of various amino acids in anther representing the pollen and stigma before, during and after pollination stages of *Solanum surattense*. Also an attempt was made to study the role of minor and major elements present in the stigma before and after pollination.

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MATERIALAND METHODS

Preparation of plant materials for loading on chromatographic paper

500 mg of each, anther and stigma of *Solanum surattense* were taken separately before, during and after pollination stages and ground using pestle and mortar in ten fold volume of 70 % ethanol. After centrifugation at 10,000 rpm for 7 min., supernatant was collected. For evaporation, supernatant was kept in petridish for 1 hr.

Preparation of solutions of known amino acids for loading on chromatographic paper

Tyrosine and phenylalanine were dissolved in 0.05 N HCl while tryptophan was dissolved in 0.05 N NaOH. Remaining amino acids were dissolved in 10 % isopropanol. The concentration of amino acids was maintained to 1 mg/ml.

Loading of samples of plant materials and known amino acids on chromatographic papers

Two chromatographic papers (each of 16" X 28" size), one each for anther and stigma, were taken. A line was drawn across each sheet leaving a margin of one inch at bottom. 27 points at the regular intervals of one inch were drawn on the paper. First three points were used for loading of plant materials of different stages while remaining points were used to load known amino acids. The materials were loaded using micropipette.

Solvent system

For the separation of amino acids, mixture containing n-butanol, glacial acetic acid and water in the ratio of 12:3:5 was used.

The chromatographic paper with loaded materials was kept in air tight chamber containing solvent for 40 hr. 0.2% ninhydrin prepared in acetone was sprayed on the paper uniformly. Hydroxyproline and proline

revealed yellow colored spots while remaining amino acids showed purple colored spots. Rf values of all the amino acids were calculated using standard formula.

For the quantitative estimations of trace elements as well as major elements in stigma Atomic absorption Spectrophotometric method was used. 500 mg of dry powdered samples of stigma (before and after pollination) were digested separately in Kjeldhal flask with triple acid i.e. concentrated HNO_3, H_2SO_4 and $HClO_4$ in the ratio of 10:4:1 at low temperature to minimize the loss of metals. The digested materials were dissolved in 0.1M HNO_3 and final volume was made to 10 ml. For control, 10 ml. of triple acid was evaporated and residue was dissolved in 0.1M HNO_3 and final volume was made to 10 ml. The samples were analysed on Atomic Absorption Spectrophotometer.

The reading of control was deducted from the sample's reading. The results were expressed as µg/g dry weight.

RESULTS

The plant *Solanum surattense*, belonging to family Solanaceae, is a very prickly, diffuse bright green perennial herb. Its flowers possess long, glabrous, oblong, lanceolate anthers which open by an apical pore. Round triporate pollen grains possess smooth surface. During *in vitro* pollen germination pollen tubes emerge from all three germ pores but only one grows and elongates later on. During the pollination entire flower bends downwardly and pollen grains come out from the anther through an apical pore and fall on stigmatic surface directly. In this way *Solanum surattense* shows self pollination. Stigmatic surface is papillate and wet type.

Amino acids in Anther and Stigma

Before anther dehiscence, out of 24 types of amino acids, 11 types were found in the anther. These were alanine, cysteine, DL-dopa, glycine, isoleucine, leucine, lysine, methionine, proline, serine and tryrosine. However, during and after anther dehiscence, 15 types of amino acids were noticed in the anther indicating the appearance of 4 new types of amino acids namely : arginine , aspartic acid, ornithine and valine (Fig. 1A; Table 1) Similarly, 11 types of amino acids were noticed in the stigma before pollination stage but16 types of amino acids are found present in the stigma during and after pollination stages indicating appearance of 5 new types of amino acids namely aspartic acid, lysine, ornithine, proline and valine (Fig. 1B; Table 2.) It is interesting to note that hydroxy proline was absent in anther but present in stigma during all three stages studied.

Major and Minor Elements in stigma before and after pollination stages

The stigma of *Solanum surattense* contains maximum amount of potassium as a major element. The amount of magnesium ranks second, while calcium is the third important major element. Comparative study of the concentration of various elements present in the stigma, before and after pollination stages in *Solanum surattense* shows considerable decrease in the major elements such as potassium (from 32885.00 to 27145.02 µg/g) and magnesium (from 2712.83 to 1952.38 µg/g) and an increase in calcium from 1806.45 to 2542.85 µg/g from before to after pollination stage (Table 3).

In the minor elements Na, Ni, Zn and Fe showed substantial decrease in the amount, while Cu, Mn, Pb, Cd and Cr revealed a considerable increase in the amount from before to after pollination stage (Table 3).

DISCUSSION

Quantitative determination of free amino acids of fresh anthers and stigma by ascending paper chromatography indicates that in *Solanum surattense*, arginin, aspartic acid, ornithine and valine are appeared in anther during and after pollination stages. These newly appeared free amino acids may play an important role in pollen germination and tube growth. Amino acids are the precursors or activators of phytohormones and growth substances [19]. In *Nicotiana tabaccum*, amongst different amino acids tested, only glutamine acid and aspartic acid and their amides stimulate pollen tube growth [20]). Swada [21] also reported that arginin and alanine promote germination of *Oryza sativa* pollen.

The increase in free arginin coincides with a marked increase in arginase activity and could play an important role in the nutrition during pollen germination [22]. Addition of aspartic acid to the sugar agar medium also caused germination of *Paris hexaphylla* pollen which otherwise did not germinate [21]. Onomo *et al*.[23] has reported that in non germinated seeds of *Cola acuminata* arginin was the major amino acid and valine was the least present, indicating high need of arginin during seed germination.

Appearance of proline and lysine in stigma of *Solanum surattense* during and after pollination stages not only supports the pollen germination and tube growth on stigmatic surface but also plays an important role in pollen-pistil interaction. Proline is utilized by growing pollen tube by being directly incorporated into the specific protein of protoplast, while excessive proline is oxidized and it becomes a source of nitrogen for the construction of new amino acids from which proteins are built during the growth of pollen tube [24], while in maize proline which represents the predominant compound in the amino acid pool of pollen grains as revealed to be effective in improving germination and tube growth [25]. Similarly, hydroxyl proline, a derivative of proline, at high concentration was found to promote tube length [26]. Thus increased amount of proline in stigma, during and after pollination, plays an important role in metabolism for energy delivery for the growth, protein synthesis and particularly as hydroxyl proline for tube length.

It can be concluded that new types of amino acids appeared in anther and stigma during and after pollination stages may: (i) play an important role in recognition reaction during pollen pistil interaction, (ii) stimulate pollen tube growth and promote tube length and (iii) become a source of nitrogen for synthesis of new amino acids.

A considerable increase in the amount of calcium while decrease in the amount of potassium and magnesium was reported in the stigma from before to after pollination stage. Similarly, the amount of Na, Ni, Zn and Fe were increased, and that of Cu, Mn, Pb, Cd and Cr were decreased in stigma after pollination.

The increase in the amount of mineral elements in stigma after pollination may be due to the transfer of pollen grains from anther to stigma. Calcium was reported to be essential for pollen germination and tube growth [4]. It establishes that polarity of pollen tube

forms a basis for pulsatile growth [13]. Magnesium and potassium are reported to be absent from pollen [27]. The presence of these major elements in stigma supports the pollen germination and tube growth [28]. As these elements are utilized in physiological activities of pollen germination and early tube growth, a decline in the amount of Mg and K is quite obvious.

Bruyn [18] has reported that copper, magnesium and zinc stimulate germination and growth of pollen in *Setaria sphacelata*. Yang et al. [15] have reported that in *Paris polyphylla* var. stenophylla when the concentrations of Zn and Mo were high, the pollen germination and tube growth were inhibited. If concentrations of these elements were low, there was no effect on germination and tube growth. However the concentration within a certain range influenced the process of pollen germination. Zinc deficiency also caused structural alterations in exine and retarded germination and tube growth in *Vigna mungo* [16].

Fe and Cu both exist in multiple redox states, readily accepting and donating electrons from their orbits. As such, Fe and Cu serve as critical cofactors for components of the electron transport chain in the mitochondria and in the chloroplast [29] indicating their importance in metabolic activities.

Based on the above results, it may be concluded that following would be the reasons for change in the amounts of major and minor elements in stigma after pollination:

- increase may be due to transfer of pollen grains carrying the elements from anther to stigma and

- decrease may be due to utilization of elements present in stigma for physiological activities required for pollen germination and tube growth.

Fig. 1 A: Chromatographic separation of free amino acids from the anther of *Solanum surratense* Burm. f. B=before, D=during, A=after pollination; 1-24= known amino acids

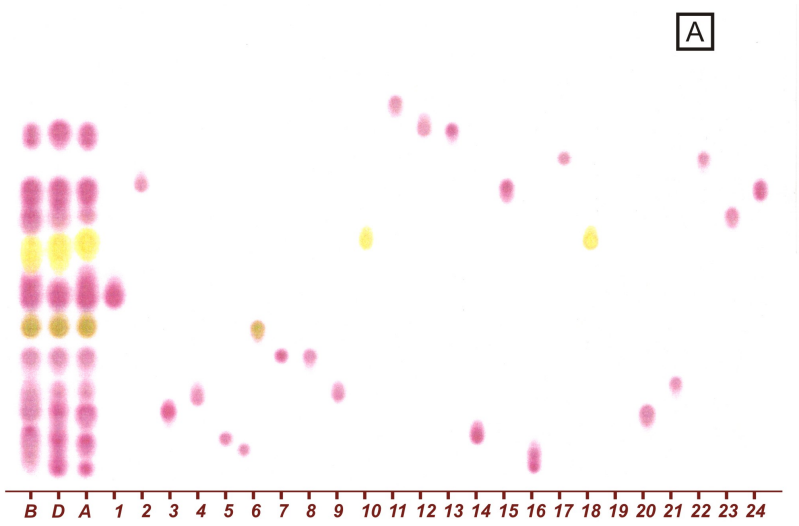

Fig. 1 B : Chromatographic separation of free amino acids from the stigma of *Solanum surratense* Burm. f. B=before, D=during, A=after pollination; 1 – 24 = known amino acids

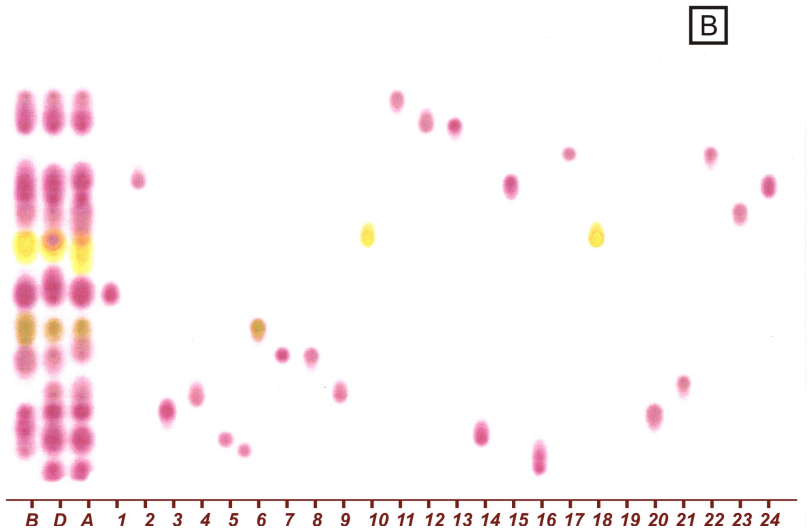

Table: 1 : Chromatographic separation of free amino acids in anther of *Solanum surattense* Burm. f.

		Chromatographic separation of free amino acids in anther of *Solanum surattense* Burm. f.		
Sr. No.	Amino acids	Before Pollination (before anther dehiscence)	During Pollination (during anther dehiscence)	After Pollination (after anther dehiscence)
1	ALANINE	+	+	+
2	AMINO-N- BUTYRIC ACID	-	-	-
3	!ARGININE HCL	-	+	+
4	!ASPARTIC ACID	-	+	+
5	CYSTEINE HCL	-	-	-
6	CYSTEINE	+	+	+
7	DL-DOPA	+	+	+
8	GLUT AMIC ACID	-	-	-
9	GLYCINE	+	+	+
10	HISTIDIN HCL	-	-	-
11	HYDROXY PROLINE	-	-	-
12	ISO-LEUCINE	+	+	+
13	NOR - LEUCINE	-	-	-
14	LEUCINE	+	+	+
15	LYSINE	+	+	+
16	METHIONINE	+	+	+
17	ORNITHIN HCL	-	+	+
18	B-PHENYL ALAN IN E	-	-	-
19	PROLINE	+	+	+
20	SERINE	+	+	+
21	THREONINE	-	-	-
22	TRYPTOPHANE	-	-	-
23	TYROSINE	+	+	+
24	VALINE	-	+	+

Table: 2 Chromatographic separation of free amino acids in stigma of *Solanum surattense* Burm. f.

Sr. No.	Amino acids	Before Pollination (before anther dehiscence)	During Pollination (during anther dehiscence)	After Pollination (after anther dehiscence)
1	ALANINE	+	+	+
2	AMINO-N- BUTYRIC ACID	-	-	-
3	!ARGININE HCL	+	+	+
4	!ASPARTIC ACID	-	+	+
5	CYSTEINE HCL	-	-	-
6	CYSTEINE	+	+	+
7	DL-DOPA	+	+	+
8	GLUT AMIC ACID	-	-	-
9	GLYCINE	+	+	+
10	HISTIDIN HCL	-	-	-
11	HYDROXY PROLINE	+	+	+
12	ISO-LEUCINE	+	+	+
13	NOR - LEUCINE	-	-	-
14	LEUCINE	+	+	+
15	LYSINE	-	+	+
16	METHIONINE	+	+	+
17	ORNITHIN HCL	-	+	+
18	B - PHENYL ALAN IN E	-	-	-
19	PROLINE	-	+	+
20	SERINE	+	+	+
21	THREONINE	-	-	-
22	TRYPTOPHANE	-	-	-
23	TYROSINE	+	+	+
24	VALINE	-	+	+

Table: 3 Comparative study of concentrations of various minor elements and major elements in before and after pollinated stigma of *Solanum surattense* Burm. f., estimated by Atomic Absorption Spectrophotometer (AS)

Elements		Concentrations of various minor elements and major elements µg/g	
		Before pollination	After pollination
Major	K	32885.00	27145.02
	Ca	1806.45	2542.85
	Mg	2712.83	1952.38
Minor	Na	1631.45	1251.29
	eu	13.47	37.18
	Mn	28.71	34.52
	Ni	32.04	10.36
	Zn	16.21	07.45
	Pb	03.00	04.12
	Fe	8.54	02.32
	Cd	00.57	01.35
	Cr	00.93	01.72

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