The Bioscan AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES 20(2): S.I (2): 195-197, 2025www.thebioscan.com

Analysis of Carbohydrates, Proteins, And Free Amino Acid Content In Pollen Of Some Crop Plants ofthe Family Poaceae.

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DOI: 10.63001/tbs.2025.v20.i02.S.I(2).pp195-197

KEYWORDS
amino acids, carbohydrates, Poaceae.

Receivedon:
22-03-2025
Acceptedon:
25-04-2025
Published on
30-05-2025

ABSTRACT

The present paper deals with the analysis of carbohydrates, protein, and free amino acid contents in pollen of some genera of the family Poaceae. The plants under investigation were *Pennisetum glaucum*,*Oryza sativa*, *Sorghum bicolor*, *Zea mays*, and *Triticum* species. The biochemical analysis of some crop plants was performed by standard methods. Detection of carbohydrates was done by the Benedict test, Fehling's test. and estimation of protein was done by the Burette test, xanthoproteic tests.Amino acids were separated and identified by the thin layer chromatographic method. Among all the species, the highest number of carbohydrates was found in *Oryza sativa*. The highest number of proteinswas found in *Pennisetum glaucum*, while the highest number of amino acids was found in*Sorghum bicolor* (15), followed by *Triticum*species(14), *Zea mays*(11), *Pennisetum glaucum* (11) and *Oryza sativa*(10). Thus, this investigation indicates that carbohydrates, proteins and essential free amino acids were present in all examined taxa of the family Poaceae. It shows that it has nutritional value.

INTRODUCTION

Pollen is a multicellular haploid gametophyte life stage of seed plants (spermatophytes) and thus it has a key function in the plant life cycle. Carbohydrates, in the form of cytoplasmic saccharides, have a vital function in the resistance of pollen to dehydration and temperature stress, as well as serving as grain wall components (cellulose) and energy reserves for germination (starch and sucrose) (Pacini et al., 2006; Bokszczanin et al., 2013). Pollen's proteins have both structural and functional roles, and have implications for both pollen-pistil and plant-pollinator interactions. Pollen proteins are an important source of dietary nitrogen for a majority of pollinators, while as enzymes they have a crucial function during pollen tube growth (Roulston et al., 2000).

Regarding the free amino acid composition of different pollen, all the essentialamino acids have been reported to be present in pollen and the total level of free amino acids is usually higher in pollen than in leaves and other tissues. It is further 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. At the same time, the concentration of all amino acids in pollen is considerably higher in the bound from than in the free fraction. (Stanley & Linskens, 1974).

2. MATERIAL & METHODS

For the analysis of carbohydrates, proteins, and free amino acid contents in pollen of some crop plants of the family Poaceae, the extraction as well as qualitative analysis was performed by standard methods. (Sadasivam et al.1992; Buzarbarua,2000).100mg of pollen powder sample was homogenised and crushed with the help of a mortar and pestle in10 ml of 70% alcohol for half an hour. Alcoholic extract was separated by centrifugation at 6000-7000 rpm and the residue was treated with a fresh portion of the same solvent and separated. The same procedure was repeated three to forth. The supernatant was collected and the volume reduced by evaporation. This extract was used to perform variousqualitative tests and TLC study for the analysis of free amino acids.

2.1 Test for carbohydrates

1. Molisch's Test: 2 drops of Molisch's reagent to about 2 ml of the test solution was mixed properly and about 1 ml of concentrated sulfuric acidwas added along the side of the tube. The purplecolour was developed due to the formation of furfural derivatives by the action of acid,indicating the presence of carbohydrates.

2. Fehling's Test: an equal amount of Fehling's (A) and (B) in a test tube was mixed well and added few drops of the test solution. Then it was boiled for a few minutes. Cupric hydroxide in Fehling's solution is reduced to cuprous oxide, and form yellow coloured ppt indicates the presence of sugar.

3. Benedict's Test:5 drops of the test solution were added to 2 ml of Benedict's reagent and boiled for a few minutes. Yellow coloured ppt is formed, which indicates the presence of sugar.

2.2 Test for proteins

1. Biurete Test: An Equal amount of test solution and 10% sodium hydroxide was mixed thoroughly, followed by 1% copper sulphate solution was added drop by drop. A violet colour is formed. This indicates the presence of protein.

2. Xanthoproteic Test: 1 ml of conc. Nitric acid was added to the test solution. Yellow colourdevelops if amino acids are present, which turn bright orange on the addition of 40% NaOH.

3. Glyoxylic Test: Ina 2 ml test solution, 2 ml of glyoxylic acid was added. After mixing well,2 ml of the conc. H_2SO_4 was added down the side of the tube. A violet ring appeared at the junction of two layers if tryptophan is present.

2.3 Test for free amino acid

1.Ninhydrin test: Asmall quantity of test solution (1 ml) was kept in the test tube and to it,1 ml of Ninhydrin reagent was added. If purple or yellow colour develops, it indicates the presence of amino acids.

2. Xanthoproteic Test: 1 ml of conc. Nitric acid was added to the test solution. Yellow colourdevelops if amino acids are present, which turn bright orange on the addition of 40% NaOH.

3. Millon's reaction: Afew drops of Millon's reagent were added to 1 ml of test solution and heated for 10 min. After cooling, 5 drops of 1% sodium nitrite solution were added. Amino acid, particularly tyrosine, was present if a brick red colour was developed.

4. Glyoxylic Test: Ina 2 ml test solution, 2 ml of glyoxylic acid was added. After mixing well,2 ml of the conc. H_2SO_4 was added down the side of the tube. A violet ring appeared at the junction of two layers if tryptophan is present.

5.Sakaguchi test: 40% NaOH solution and test solution were mixed (1:3) in a test tube and 2 drops of 1% alpha naphthol in alcohol and a few drops of bromine water were added to the mixture. The development of red colour confirms the presence of arginine in the test solution.

Quantitative analysis

Quantitative analysis of the free amino acid by thin layer chromatography was carried out on a silica gel-coated glass plate.the plate was activated at 100- 110 °c before spotting. The spotting was carefully done with the help of capillary tubes. and the solvent was butanol, acetic acid, and water in a 4:1:1 ratio. Then, 0.3% ninhydrin in butanol containing 3 ml of acetic acid solution was used for the detection of amino acids. It was sprayed on the plate. the spots of amino acids were developed after heating the plate at 100- 110 °c for 5 to 10 min. the spots were identified by calculating the Rf values. And comparing with the standard ones.

Table 1: Qualitative analysis of carbohydrates by preliminary biochemical screening of pollen grains of studied plants

Test	P.G.	O.S.	S.B.	Z.M.	T.S.
Molisch's Test	+	+	+	+	+
Fehling's Test	+	+	+	+	+
Benedicts Test	+	+	+	+	+

Table 2: Qualitative analysis of protein by preliminary biochemical screening of pollen grains of studied plants

Test	P.G.	O.S.	S.B.	Z.M.	T.S.
Burette Test	+	+	+	+	+
Xanthoproteic Test	+	+	+	+	+
Glyoxylic Test	+	+	+	+	+

Table 3: Qualitative analysis of free amino acids by preliminary biochemical screening of pollen grains of studied plants

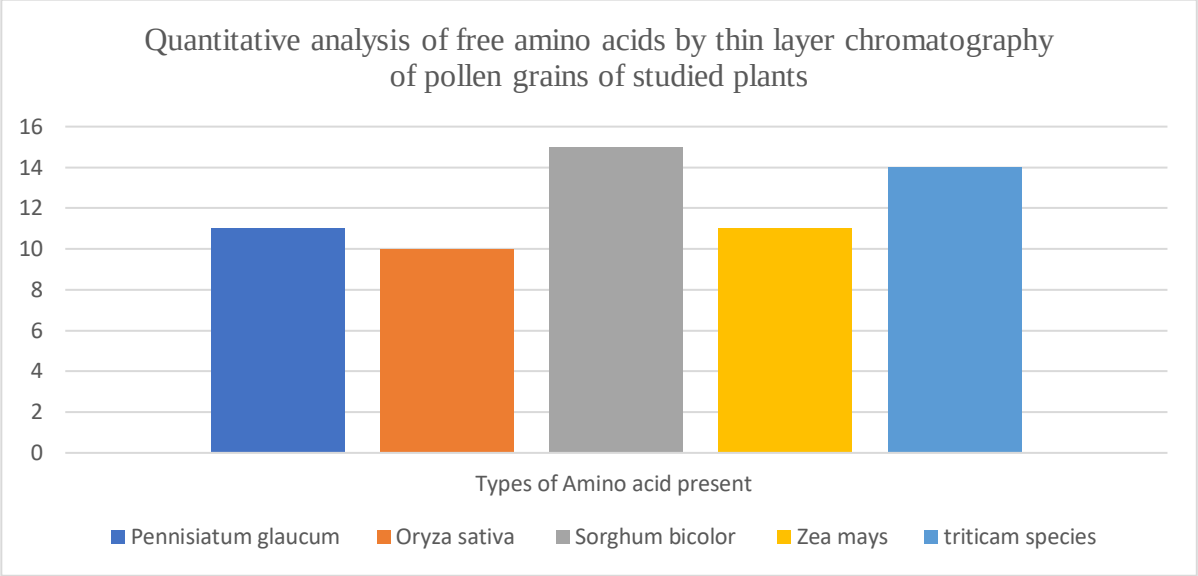
Test	P.G.	O.S.	S.B.	Z.M.	T.S.
Ninhydrin test	+	+	+	+	+
Xanthoproteic Test	+	+	+	+	+
Millon's reaction	-	-	-	-	-
Glyoxylic Test	+	+	+	+	+
Sakaguachi test	-	+	+	+	+

Table 4: Quantitative analysis of free amino acids by thin layer chromatographyof pollen grains of the studied plants

Sr.no.	Amino Acids	P.G.	O.S.	S.B.	Z.M.	T.S.
1	Alanine	+	+	+	-	-
2	Arginine	-	+	+	+	+
3	Aspartic acid	-	-	+	+	-
4	Cysteine	-	-	-	-	-
5	Glutamic acid	+	-	+	-	+
6	Glutamine	-	-	+	-	+
7	Glycine	-	-	+	-	+
8	Histidine	+	+	+	+	+
9	Isoleucine	+	+	+	+	+
10	Leucine	+	+	+	+	+
11	Lysine	-	-	-	-	-
12	Methionine	+	-	-	-	+
13	Ornithine	+	+	+	+	+
14	Phenylalanine	-	-	-	-	-
15	Proline	+	+	+	+	+
16	Serine	+	+	+	+	+
17	Threonine	+	+	+	+	+
18	Tryptophan	+	+	+	+	+
19	Tyrosine	-	-	-	-	-
20	Valine	-	-	+	+	+
Total		11	10	15	11	14

Note:

'-' = Absent + = Present P.G.= *Pennisetum glaucum* O.S.= *Oryza sativa* S.B.= *Sorghum bicolor*Z.M.= *Zea mays* T.S.= *Triticum species*



RESULTS

The carbohydrates, proteins and free amino acids of the pollen grain of five investigated taxa are presented in Tables 1 to 3. The qualitative tests for carbohydrates, like Molisch's test, Fehling's test, and Benedict's test., have shown positive results in all taxa. The qualitative tests for protein, like the Burette test, Xanthoproteic test and Glyoxylic test. Show positive results in all taxa. And test for free amino acids like Glyoxylic test, xanthoproteic test, and Sakaguchi test. Have shown positive results and confirmed the presence of amino acids like tryptophan and arginine.

The TLC result presented in TableNo. 4 indicates the presence of various amino acids in different pollen grains of the studied plants.

DISCUSION

In the present investigation for analysis of free amino acids, proline and histidine were found in pollen of all investigated taxa. Tupy in 1964 reported that the histidine-proline quotient in pollen represents an important criterion of pollen fertility.

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 conditions, as well as with storage and handling patterns. (Iwanami 1959). Results of the present work showed that at least 10 amino acids were found in all taxa studied.

CONCLUSION

The present investigation indicates that carbohydrates, proteins and essential free amino acids were present in all examined taxa of the family Poaceae. It shows that it has nutritional value.

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