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RESEARCH ARTICLE

# Qualitative analysis of amino acids in pollen and nectar of onion (*Allium cepa* L.) cv. Bhima Super

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## Abstract

The present investigation was carried out to qualitatively identify amino acids present in the nectar and pollen of onion (*Allium cepa* L.) cv. Bhima Super, using paper chromatography and to evaluate their potential ecological role in pollination. Onion is a cross-pollinated crop that depends heavily on insect pollinators, particularly honey bees (*Apis mellifera*), for effective seed production. Floral extracts were prepared from fresh nectar and pollen samples collected during peak flowering and subjected to paper chromatography using Whatman No. 1 filter paper and a solvent system of *n*-butanol:acetic acid:water (4:1:5 v/v). After solvent migration, the paper strips were sprayed with 0.2 % ninhydrin and heated at 60 °C for spot development. Amino acids were identified by comparing the Retention factor (Rf) values of the samples with those of standard amino acids. A total of 9 amino acids were identified: valine, leucine, isoleucine, threonine, glycine, serine, alanine, glutamic acid and aspartic acid. Proline and phenylalanine were additionally detected in nectar, both known to influence pollinator attraction, energy metabolism and floral scent production. The results confirm the biochemical richness of Bhima Super onion nectar and pollen, suggesting their potential role in enhancing pollinator visitation and ultimately improving pollination efficiency and seed yield. Paper chromatography proved to be a simple and cost-effective method for preliminary amino acid profiling in floral components. However, further quantitative analysis using advanced chromatographic techniques is recommended to determine the precise ecological relevance of individual amino acids in pollination biology.

**Keywords:** amino acids; Bhima Super; paper chromatography; phenylalanine; pollen; pollination

## Introduction

Pollination plays an indispensable role in the reproductive success of flowering plants and the sustainability of agroecosystems, particularly in crops that rely on biotic vectors for fertilization. Onion (*Allium cepa* L.), an economically significant crop cultivated worldwide for bulbs and seeds, is a staple ingredient in diverse cuisines and represents one of the highest-value vegetable crops, with annual global production exceeding 100 million tonnes, is predominantly cross-pollinated and therefore heavily dependent on insect-mediated pollination for successful seed set (1). Among insect pollinators, honey bees (*Apis mellifera*) are recognized as the principal agents contributing to onion pollination due to their floral constancy and foraging efficiency (2). However, the frequency and effectiveness of pollinator visitation are influenced not just by floral morphology but also by the biochemical composition of floral rewards-namely nectar and pollen (3, 4). Nectar and pollen are the primary sources of nutrition for pollinators, supplying energy-rich sugars and essential amino acids that are vital for their growth, development and reproduction (5). Amino acids, although present in much smaller quantities compared to sugars, serve crucial functions in modulating pollinator behavior-both by influencing the taste and palatability of floral rewards as well as through their metabolic impacts on insect physiology.

Essential amino acids such as valine, leucine, isoleucine and threonine are not synthesized by insects and must be acquired through diet, making their presence in nectar and pollen highly beneficial to pollinators (6). Non-essential amino acids like glycine, serine and glutamic acid, though not nutritionally obligatory, influence nectar palatability and can act as phagostimulants, enhancing the attractiveness of flowers and increasing the duration of visits by pollinators (7, 8). For example, proline accumulation increases in plants subjected to drought, salinity or extreme temperatures, helping them maintain osmotic balance and protect cellular structures during stress. In honey bees (*Apis mellifera*), proline is a preferred energy source during flight and is metabolized rapidly during initial foraging activity (9), though its importance may vary among bee species. Its presence in floral nectar is associated with increased pollinator preference and improved foraging efficiency (10). Similarly, phenylalanine, an aromatic amino acid, acts as a precursor for floral scent compounds and contributes to scent-mediated pollinator attraction (11). The balance and diversity of these amino acids in nectar and pollen can significantly influence pollinator visitation patterns, foraging rates and ultimately, pollination success and seed yield in crops like onion. Despite the established significance of floral biochemical traits, there is limited information on the qualitative amino acid composition of nectar and

pollen in onion cultivars. Most available studies focus on nectar sugar profiles or pollinator activity patterns, while fewer address the amino acid diversity in floral rewards. The Bhima Super cultivar, a widely adopted variety of onion in India, is known for its superior bulb and seed yield performance under field conditions. Characterizing its floral biochemistry, especially the amino acid profile, could provide insights into its attractiveness to pollinators and inform strategies to enhance pollination efficiency.

Paper chromatography is a simple, cost-effective method for the preliminary qualitative analysis of amino acids in plant materials. Though not quantitative, it allows for the reliable identification of amino acids based on retention factor (Rf) values when compared with known standards (12). This method is particularly useful in field-based studies or in resource-limited settings where advanced instrumentation like High-performance liquid chromatography (HPLC) or liquid chromatography-tandem mass spectrometry (LC-MS/MS) may not be readily available.

In this context, the present study was undertaken to qualitatively identify amino acids present in the nectar and pollen of Bhima Super onion, using paper chromatography and to examine their potential role in influencing pollinator visitation. By bridging plant biochemistry and pollinator ecology, this research aims to enhance our understanding of floral traits that drive pollination success in onion and support future breeding and agronomic practices that are pollinator-friendly.

## Materials and Methods

### Plant material and sample collection

The study was conducted at ICAR-Directorate of Onion and Garlic Research (DOGR), Rajgurunagar. The center is located at 18.32° N (latitude) and 73.51° E (longitude) at 553.8 m above mean sea level with a mean temperature around 29 °C and an annual mean rainfall of 669 mm. The experiment was conducted in the late *Kharif* season of 2024-25 using the Bhima Super onion during its peak flowering stage under cage field conditions to ensure controlled pollination and minimal external contamination. Healthy umbels bearing fully opened florets were selected from well-maintained plants grown within insect-proof cages.

Nectar was collected during the early morning hr (07:00-09:00 hr) using sterilized microcapillary tubes, a time known for peak nectar secretion due to favorable turgor pressure and reduced evaporative losses (8, 13). The use of microcapillary tubes allowed for precise extraction without damaging the floral tissues or introducing contaminants. To ensure a representative biochemical profile and minimize variability, nectar from multiple umbels was pooled, a common approach in floral biochemical studies when individual sample volumes are limited or inter-floral variation is significant (14, 15).

Bee-collected pollen was obtained using pollen traps attached to the hive entrances of *A. mellifera* colonies placed inside the cages. As forager bees visited flowers and returned to the hive, the traps collected pollen pellets dislodged from their hind legs. These pellets, commonly referred to as bee-collected pollen or bee pollen, are packed by bees using their leg combs and transferred to the pollen baskets (16). Freshly collected pollen was used for analysis before any hive-based fermentation occurred. The inclusion of cage conditions ensured accurate tracking of floral resources and pollinator interaction within a semi-controlled environment.

### Reagents and materials

Whatman No. 1 chromatographic-grade filter paper was used as the stationary phase for paper chromatography (17). The solvent system consisted of *n*-butanol, acetic acid and distilled water in a 4:1:5 (v/v) ratio and the upper organic phase was used for chromatographic development. Standard amino acids (Sigma-Aldrich, ≥ 99 % purity) were identification and Rf value calibration. A 0.2 % ninhydrin solution prepared in acetone served as the detecting reagent. Micropipettes or capillary tubes were employed for spotting the samples. Chromatographic development was carried out in a glass chamber and color development was performed using a hot-air oven maintained at 60 °C.

### Preparation of extracts

#### Nectar extract

Collected nectar was diluted with an equal volume of distilled water and centrifuged at 3000 rpm for 5 min to remove any particulates. The clear supernatant was used for analysis. The nectar was pooled from multiple umbels to minimize intra-plant variation and obtain a representative biochemical profile (18). For extraction, the pooled nectar was diluted in a 1:1 ratio with distilled water to reduce viscosity and improve chromatographic resolution (19). The solution was then centrifuged at 3000 rpm for 5 min to remove pollen grains, waxy particles and other impurities (20). The supernatant was collected into sterile microcentrifuge tubes and stored at 4 °C until further analysis.

#### Pollen extract

Approximately 100 mg of bee-collected pollen was homogenized in 2 mL of 80 % ethanol. The homogenate was incubated at 60 °C in a water bath for 15 min and subsequently filtered using Whatman No. 1 filter paper. The resulting filtrate served as the working solution for chromatographic analysis (21, 22).

#### Paper chromatography

Amino acid profiling was performed using thin-layer chromatography (17). Rectangular strips (20 × 15 cm) of Whatman No. 1 filter paper were used as the stationary phase. A baseline was drawn 2 cm from the bottom. Using a capillary tube or micropipette, 2-3 µL of each sample extract and standard amino acids were spotted onto the baseline. Spots were air-dried between applications to concentrate the sample.

The prepared chromatograms were placed in a chromatographic chamber saturated with the solvent system (*n*-butanol:acetic acid:water, 4:1:5 v/v). The solvent was allowed to ascend up to 15 cm from the baseline. After development, chromatograms were air-dried and uniformly sprayed with 0.2 % ninhydrin reagent in acetone. Color development was completed by incubating the strips in a hot air oven at 60 °C for 10 min.

### Rf value calculation and compound identification

The distance travelled by each sample spot and the solvent front was measured in cm. Retention factor (Rf) values were calculated using the formula:

Rf = distance travelled by the compound / distance travelled by the solvent front (Eqn. 1)

The obtained Rf values of nectar and pollen samples were compared with those of known standard amino acids under identical chromatographic conditions for compound identification.

## Results

### Amino acid identification in pollen (paper chromatography Rf matches)

The chromatographic profiling of bee-collected pollen revealed in Fig. 1 illustrates representative paper chromatograms with the migration of 9 pollen amino acids whose calculated Rf values closely aligned with standard references, supporting accurate qualitative identification. Serine was detected at an Rf of 0.30 (spot distance 3.0 cm over a 10.0 cm solvent front), matching the standard Rf of 0.30 and indicating a consistent presence in pollen. Glycine exhibited an Rf of 0.25 (2.5/10.0), in close agreement with the standard 0.26, reinforcing its role as a common non-essential amino acid in floral rewards. Threonine, an essential amino acid, was observed at an Rf of 0.45 (4.5/10.0) against a standard of 0.44 and alanine at 0.58 (5.8/10.0) versus 0.60, both within acceptable experimental tolerance for paper chromatography. Valine appeared at an Rf of 0.68 (6.8/10.0), near the reference 0.71, indicating reliable detection of branched-chain amino acids in pollen.

The acidic amino acids were also present, such as glutamic acid at 0.17 (1.7/10.0) versus 0.18 and aspartic acid at 0.52 (5.2/10.0), matching perfectly with 0.52. Among essential amino acids, leucine and isoleucine were evident with higher Rf values, at 0.82 (8.2/10.0; standard 0.84) and 0.75 (7.5/10.0; standard 0.76) respectively, confirming a nutritionally diverse pollen amino acid profile beneficial for pollinators (Table 1). Collectively, these data show strong concordance between calculated and standard Rf values (absolute deviations typically ≤ 0.03), validating the method and indicating consistent amino acid identities in the Bhima Super pollen matrix. Nine amino acids were detected in the pollen matrix, including glutamic acid, glycine, serine, threonine, alanine, valine, leucine, isoleucine and aspartic acid. Of these, isoleucine and aspartic acid were unique to pollen, absent from nectar, indicating that pollen delivers specific nutritional compounds essential for pollinator development and health. The remaining 7 amino acids, including glutamic acid, glycine, serine, threonine, alanine, valine and leucine formed a core set shared with nectar, underscoring their fundamental role in supporting pollinator nutrition.

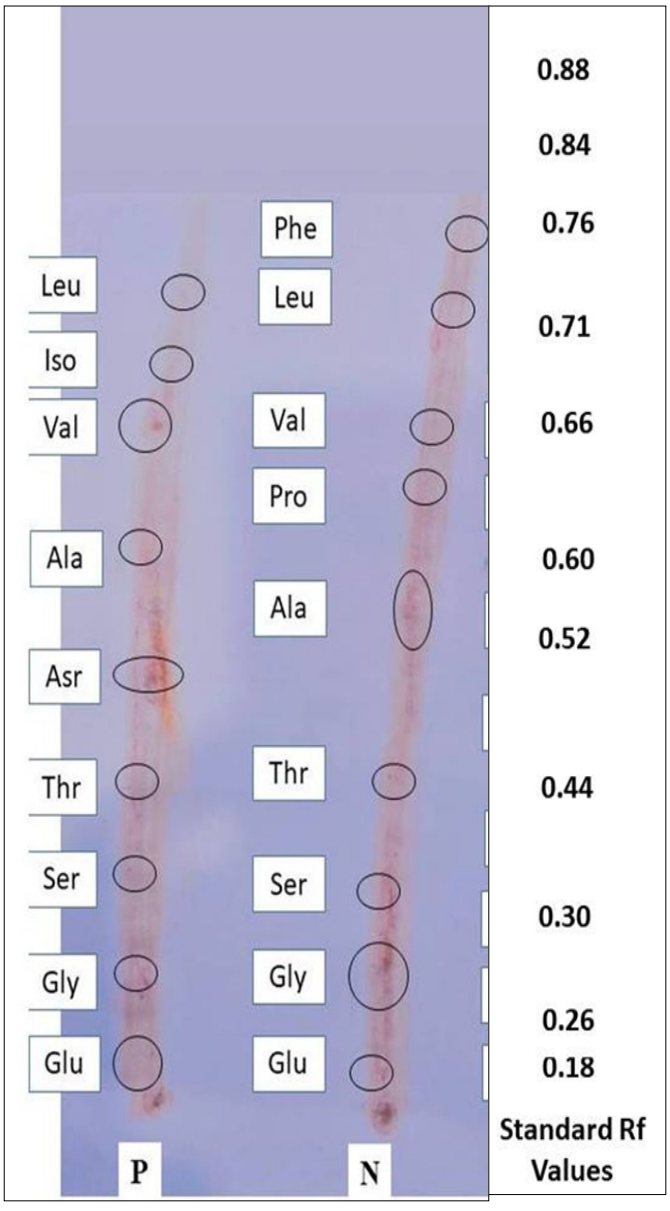


**Fig. 1.** TLC plate showing spots of amino acids separated from pollen and nectar sample.

### Amino acid identification in nectar (paper chromatography Rf matches)

Fig. 1 illustrates representative paper chromatograms with the migration of 9 nector's amino acid spots relative to the solvent front, alongside the formula used for Rf calculation, demonstrating a distinct yet overlapping amino acid spectrum compared to pollen. Glutamic acid showed an exact match at Rf 0.18 (1.8/10.0) and glycine also matched precisely at 0.26 (2.6/10.0), confirming the presence of key non-essential amino acids that modulate nectar taste. Serine was detected at Rf 0.33 (3.3/10.0), closely aligning with its standard 0.32, while threonine matched its standard at 0.44 (4.4/10.0), highlighting essential amino acids available to foraging bees. Alanine appeared at 0.57 (5.7/10.0), near the 0.60 standard and proline, important for bee flight metabolism, was clearly present at 0.66 (6.6/10.0), an exact match to its reference value.

Valine was identified at 0.73 (7.3/10.0) with minor deviation from the 0.71 standard and leucine at 0.83 (8.3/10.0), very close to 0.84, further indicating branched-chain amino acids in nectar. Notably, phenylalanine (aromatic) was present with a high Rf of 0.89 (8.9/10.0) against a standard of 0.88, indicating a potential link with floral scent biosynthetic pathways (Table 2). The overall agreement with standards underscores the reliability of the qualitative identifications and shows that Bhima Super nectar includes both phagostimulatory and metabolically relevant amino acids. Nectar exhibited a slightly different profile, with 9 amino acids detected as well. Core amino acids shared with pollen included glutamic acid, glycine, serine, threonine, alanine, valine and leucine. Additionally, nectar uniquely contained proline and phenylalanine, which were absent in pollen. Proline is linked to bee energetics as a major energy source during flight, while phenylalanine contributes to floral aroma and plays a role in pollinator attraction via scent cues.

## Discussion

The primary objective of this study was to qualitatively identify amino acids present in the nectar of Bhima Super onion using paper chromatography to explore their ecological significance in relation to pollination. The analysis confirmed the presence of 9 amino acids, serine, glycine, threonine, alanine, valine, glutamic acid, aspartic acid, leucine and isoleucine by comparing calculated Rf values with those of known standards. These findings are in agreement with earlier studies reporting diverse amino acid profiles in floral nectars and pollens across various angiosperm species, which are crucial

**Table 1.** Identification of amino acids in pollen extract of *Allium cepa* L. cv. Bhima Super, using paper chromatography

| S. No. | Spot distance (cm) | Solvent front (cm) | Calculated Rf | Standard Rf | Identified amino acid | Amino acid type† | Functional role in pollination |
|---|---|---|---|---|---|---|---|
| 1 | 3.0 | 10.0 | 0.30 | 0.30 | Serine | Non-essential | Enhances nectar palatability and floral scent perception |
| 2 | 2.5 | 10.0 | 0.25 | 0.26 | Glycine | Non-essential | Acts as a phagostimulant and improves taste for pollinators |
| 3 | 4.5 | 10.0 | 0.45 | 0.44 | Threonine | Essential | Supports insect development and nutritional requirements |
| 4 | 5.8 | 10.0 | 0.58 | 0.60 | Alanine | Non-essential | Contributes to floral nectar diversity |
| 5 | 6.8 | 10.0 | 0.68 | 0.71 | Valine | Essential | Important for honey bee growth and protein synthesis |
| 6 | 1.7 | 10.0 | 0.17 | 0.18 | Glutamic acid | Non-essential | Enhances pollinator response and osmotic balance |
| 7 | 5.2 | 10.0 | 0.52 | 0.52 | Aspartic acid | Non-essential | Influences nectar taste and amino acid profile |
| 8 | 8.2 | 10.0 | 0.82 | 0.84 | Leucine | Essential | Key for insect metabolism and pollinator attraction |
| 9 | 7.5 | 10.0 | 0.75 | 0.76 | Isoleucine | Essential | Improves nutritional quality of nectar/pollen |

† Based on essentiality for insect nutritional requirements.

**Table 2:** Identification of amino acids in nectar extract of Allium cepa L. cv. Bhima Super using thin layer paper chromatography

| S. No. | Spot distance (cm) | Solvent front (cm) | Calculated Rf | Standard Rf | Identified amino acid | Amino acid type† | Functional role in pollination |
|---|---|---|---|---|---|---|---|
| 1 | 1.8 | 10.0 | 0.18 | 0.18 | Glutamic acid | Non-essential | Improves nectar osmolality and bee attraction |
| 2 | 2.6 | 10.0 | 0.26 | 0.26 | Glycine | Non-essential | Stimulates foraging; enhances taste appeal |
| 3 | 3.3 | 10.0 | 0.33 | 0.32 | Serine | Non-essential | Promotes metabolic stimulation in pollinators |
| 4 | 4.4 | 10.0 | 0.44 | 0.44 | Threonine | Essential | Crucial for larval development and protein balance |
| 5 | 5.7 | 10.0 | 0.57 | 0.60 | Alanine | Non-essential | Adds complexity to nectar taste profile |
| 6 | 6.6 | 10.0 | 0.66 | 0.66 | Proline | Non-essential (but nectar-preferred) | Major phagostimulant; increases bee visitation |
| 7 | 7.3 | 10.0 | 0.73 | 0.71 | Valine | Essential | Required for bee health and brood rearing |
| 8 | 8.3 | 10.0 | 0.83 | 0.84 | Leucine | Essential | Enhances nutritional value of nectar |
| 9 | 8.9 | 10.0 | 0.89 | 0.88 | Phenylalanine (aromatic) | Essential (aromatic) | Acts in bee learning/memory; enhances flower recognition |

† Based on essentiality for insect nutritional requirements.

nutritional resources for pollinators (5, 8, 14). The presence of essential amino acids such as valine, leucine, isoleucine and threonine is particularly important, as these are required for the physiological development and reproductive fitness of insect pollinators, especially honey bees (*Apis mellifera*) (6, 23). Additionally, non-essential amino acids, including glycine, serine and glutamic acid, have been shown to enhance nectar palatability and act as phagostimulants, thereby improving flower attractiveness and potentially increasing foraging activity (7, 15, 24). These biochemical constituents influence not only the nutritional value of the nectar but also behavioral parameters such as foraging duration, visitation frequency and pollination efficiency all vital for successful onion seed production, which relies heavily on insect-mediated pollination (1, 25). Furthermore, the use of paper chromatography proved to be an effective, low-cost and accessible technique for amino acid identification in plant extracts, although its qualitative nature presents limitations in terms of precision and sensitivity.

The results also highlighted the presence of amino acids with specific ecological functions. The detection of proline, for instance, is noteworthy as it enhances energy metabolism and flight performance in bees, particularly under thermally challenging conditions (26, 27). Proline is also considered one of the most attractive nectar amino acids for honey bees and plays a role in stress response and floral resource quality. Bhima Super onion is primarily recognized for its high yield and adaptability, however, there is limited published information regarding any distinct floral scent profiles compared to other onion cultivars. Likewise, the identification of phenylalanine, an aromatic amino acid, is of ecological importance due to its role as a precursor for floral scent compounds that aid in long-distance pollinator attraction (28, 29). Amino acids such as alanine, glycine and threonine also contribute to nectar flavor and metabolic pathways, potentially enhancing the constancy and diversity of pollinator visitation (9). The amino acid diversity observed in the Bhima Super, onion cultivar aligns with previous studies on amino acid-rich nectar, where a positive correlation was established with increased pollinator visitation, improved pollination efficiency and higher seed yield (3, 30-32). However, a key limitation of the current approach lies in its inability to quantify the amino acid concentrations. Future investigations should employ high-performance techniques such as HPLC or LC-MS/MS for more precise quantification, which would allow deeper insight into the functional roles of nectar amino acid profiles in pollinator attraction and plant reproductive success (33, 34).

## Conclusion

The study successfully demonstrated the presence of essential and non-essential amino acids in total, 11 amino acids were qualitatively detected across the nectar and pollen of Bhima Super onion using thin layer paper chromatography as summarized in Table 3. The detection of amino acids such as valine, leucine, isoleucine, proline and phenylalanine highlights their potential role in enhancing pollinator attraction and pollination efficiency. These findings underscore the ecological significance of nectar composition in influencing pollinator behavior and improving seed yield in onion. While the qualitative approach provided valuable insights, future research should focus on quantitative analysis for a deeper understanding of plant-pollinator biochemical interactions.

**Table 3.** Amino acids present in bee pollen and nectar

| S. No. | Amino acid identified | Detected in pollen | Detected in nectar |
|---|---|---|---|
| 1 | Glutamic acid | ✓ | ✓ |
| 2 | Glycine | ✓ | ✓ |
| 3 | Serine | ✓ | ✓ |
| 4 | Threonine | ✓ | ✓ |
| 5 | Alanine | ✓ | ✓ |
| 6 | Valine | ✓ | ✓ |
| 7 | Leucine | ✓ | ✓ |
| 8 | Isoleucine | ✓ | – |
| 9 | Aspartic acid | ✓ | – |
| 10 | Proline | – | ✓ |
| 11 | Phenylalanine (aromatic) | – | ✓ |

Note: (✓): amino acid present and (-); amino acid absent

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## Authors' contributions

PTR contributed to conceptualization, data curation, formal analysis, investigation, methodology, statistical analysis, writing of the original draft, review and editing. DSA & VK contributed to data curation, formal analysis, investigation, methodology and writing of the original draft. ARP & MP contributed to statistical analysis and writing, review and editing.

## Compliance with ethical standards

**Conflict of interest:** The authors have no conflicts of interest to declare that are relevant to the content of this article.

**Ethical issues:** None

### Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT in order to improve readability and language of the work. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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