



Comparative Analysis of Amino Acid and Sugar Profiles in Broad Beans (*Vicia faba*) and Alfalfa (*Medicago sativa*): Implications for Suitability as Host Plants for Sap-sucking Insects Like Pea Aphids

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Author's contribution

The sole author designed the experiment, gathered data, conducted the analysis, and drafted the manuscript.

Article Information

DOI: <https://doi.org/10.9734/ijpss/2025/v37i95753>

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://pr.sdiarticle5.com/review-history/144447>

Original Research Article

Received: 15/07/2025

Accepted: 20/09/2025

Published: 23/09/2025

ABSTRACT

Pea aphids need amino acids for growth and reproduction, and sugars for energy. Broad beans (*Vicia faba*) and alfalfa (*Medicago sativa*), their common host plants, may offer different amino acid and sugar profiles, which can impact aphid rearing conditions. Thus, this study examined the quantity of these two key nutritional factors in the leaf samples of broad beans and alfalfa. The study was conducted at the college of plant protection, Northwest A& F University, Yangling, China. Linear Ion Trap mass spectrometer with Liquid chromatography separations were used to analyze

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the two nutrients in the leaf samples. The results showed that *V. faba* had higher concentrations of certain amino acids compared to *M. sativa*. In *M. sativa*, the most abundant amino acids were non-essential, including asparagine (36.03%), glutamic acid (17.37%), and serine (11.92%). Similarly, in *V. faba*, the most abundant amino acids were also non-essential, including asparagine (60%), tyrosine (7.08%), and glutamic acid (3.91%). The most abundant essential amino acids in *M. sativa* were threonine (31.28%), leucine (16.1%), and valine (15.72%), while in *V. faba*, they were histidine (48.4%), lysine (14.04%), and valine (9.7%). Essential amino acids contributed 9.65% to the total amount of amino acids in *M. sativa* and 12.35% in *V. faba*. The most varied essential amino acids in the two plants were histidine, lysine, and phenylalanine. The total concentration of fructose, glucose, and sucrose differed significantly between the two plants, with individual sugars in *V. faba* being significantly higher than those in *M. sativa*. Moreover, the analysis revealed that both total amino acids and sugars were significantly higher in *V. faba* than in *M. sativa*. These findings suggest that the broad bean may be a more suitable host plant for rearing pea aphids than alfalfa.

Keywords: Amino acids; sugars; pea aphid; broad beans; alfalfa; rearing; suitable host plant.

1. INTRODUCTION

Sap-sucking insects such as pea aphids are economically important pests that injure a wide range of crops. They negatively affect their host plants in many ways. First, as phloem feeders, they suck nutrients essential for plant growth and reproduction for their own benefit. Second, during feeding, they inject saliva into plant tissues, which can have phytotoxic effects. Third, aphids are vectors of numerous plant viruses, nearly 50% of all insect-borne viruses are transmitted by aphids. Fourth, the honeydew they secrete promotes the growth of sooty mold fungi on leaf surfaces, which interferes with photosynthesis and overall plant health (Dedryver et al., 2010). Despite these negative impacts on crops, aphids have relatively weak immune systems (Gerardo et al., 2010; Martin et al., 2021, Martin et al., 2023). This paradox has attracted interest from entomologists globally, who aim to understand how aphids survive and thrive in such hostile environments, especially within the nutrient rich but defensive plant phloem. To explore these questions, aphids must be reared under controlled laboratory conditions, which requires a suitable host plant that supports their normal growth and reproduction. Providing an optimal food source is therefore critical for maintaining healthy aphid populations in experimental setups (Malka et al., 2020).

Broad beans (*Vicia faba*) and alfalfa (*Medicago sativa*) are commonly used host plants for laboratory rearing of pea aphids. However, it remains unclear which of the two offers superior nutritional support. Although both plants are used, there is limited comparative information on their nutritional profiles specifically, the composition of amino acids and sugars in the leaves, which are the primary feeding

sites for aphids. Amino acids and sugars are key nutrients that directly affect aphid physiology (Karley et al., 2002; Clissold et al., 2015; Nalam et al., 2019). While other factors such as water content, lipids, and secondary metabolites may also influence phloem sap quality, amino acids and sugars are considered the most critical (Sandström & Pettersson, 1994; Sandström, 2000; Shelke et al., 2024). The quality and composition of these nutrients largely determine the suitability of a host plant for aphid development and reproduction (Karley et al., 2002).

The phloem sap of plants typically contains high concentrations of sugars and relatively low levels of amino acids (Sandström et al., 1994; Dixit et al., 2013; Nalam et al., 2019). Amino acids are essential nutrients for pea aphids and are closely associated with their population growth, survival, and reproductive success (Sandström et al., 1994; Weibull et al., 1990; Awmack et al., 2002). Research has shown that plants with lower total amino acid concentrations tend to be more resistant to aphid infestation compared to those with higher concentrations (Maltais et al., 1957). Additionally, stressed plants often exhibit altered nutritional profiles, which can enhance their defenses against aphids (Xie et al., 2020). Overall, the nutritional quality of host plants plays a crucial role in shaping plant insect interactions (Cao et al., 2016). Therefore, assessing the levels of sugars and amino acids in host plants is important when selecting suitable plant species for rearing aphids in laboratory studies. This study examined the amino acid and sugar content in the leaves of broad beans and alfalfa. Although other plant compounds such as secondary metabolites are also present and potentially influential, sap-sucking insects like pea aphids have evolved mechanisms to tolerate

or even sequester these compounds as a defense against predators. As a result, secondary metabolites are less likely to interfere with aphid feeding compared to primary nutrients like amino acids and sugars. Sugars and amino acids account for approximately 90% of the dry matter in phloem sap and are the key nutritional components supporting aphid development (Cao et al., 2016).

The information contained in this article therefore provides valuable insights for researchers working with sap-sucking insects such as aphids, helping them to select the most suitable host plants for their experiments. Additionally, the findings may assist farmers in choosing crop species that are naturally less susceptible to sap-sucking pests, thereby promoting more sustainable pest management practices.

2. MATERIALS AND METHODS

2.1 Plants

Broad bean (*Vicia faba*) and alfalfa (*Medicago sativa*) seeds were purchased from ShouHe Seed Industry in Shandong, China. In the laboratory, broad bean seeds were soaked in water for three days and then planted in plastic pots (16.5 cm × 12 cm) containing a soil mixture of sand, humus, and black loamy soil in a 1:3:3 ratios. The pots were kept at room temperature (25°C) until the seeds germinated. After germination, the seedlings were transplanted into small plastic pots (9.7 cm × 8.5 cm), with one seedling per pot. Seedlings at the four-leaf stage were used for the extraction of amino acids and sugars. Alfalfa seeds were also planted in plastic pots (16.5 cm × 12 cm) containing the same soil mixture used for the broad beans and watered with tap water as needed. Seedlings at 2-3 weeks after germination were then transplanted into small plastic pots (9.7 cm × 8.5 cm), with four seedlings in each pot. Seedlings at the 5-6 leaf stage were used for extracting amino acids and sugars.

2.2 Extraction and Analysis of Free Amino Acids and Sugars from the Broad Bean and Alfalfa Leaves

Amino acids and sugars were extracted separately from whole leaves of alfalfa and broad bean according to Thiele et al. (2008), Liu et al. (2016), Cao et al. (2018), and Liu et al. (2020). Briefly, when alfalfa and broad bean grew to 4-6 leaf stage, the 2nd and 3rd fresh leaves from the top of the plant were cut (Liu et al., 2016) and

weighed (about 50mg). Each weighed leaf sample was added into a 1.5mL Eppendorf tube containing 200µL of 50mM of 37% pure Hydrochloric acid (HCL) and ground fully using a tissue grinder. 800 µL of 50mM of HCL was then added to each grounded sample, mixed well, and centrifuged (Hitachi, CF 16RXII, Tokyo, Japan) at 12000g for 10 minutes. The supernatant filtered through 0.22µm syringe filters into analysis bottles (Liu et al., 2016). Nine (9) biological repeats were performed for each plant. The filtered samples were analyzed for amino acids and sugars by LTQ XL™ Linear Ion Trap mass spectrometer (Thermo Scientific, Waltham, MA, USA). For amino acids, Liquid chromatography separations were carried out with Intersil OSD-3 C18 Column (250 mm × 3.0 mm; GL Sciences Inc ; Japan). The elution was performed by applying a three-step gradient, A 100% for 8 min, 0–80% B linear for 1 min, 80% B for another 5 min, and 0–80% A linear for 1 minute, holding the system at 100% A for 8 minutes (Liu et al., 2016). Two Mobile phases were used, 'A' was an aqueous solution containing 5% acetonitrile and 0.1% formic acid, and 'B' was 100% acetonitrile with 0.1% formic acid. The flow rate was set at 260µL/min. Mass spectrometer worked in the positive electrospray ionization (ESI) mode, and the spray voltage was set at 4.5 Kv while the ion transfer capillary temperature was set at 275°C. Masses of precursor, product ions, and collision energy are listed in Table 1. The data were processed using Xcalibur 2.1 software (Thermo Scientific, Waltham, MA, USA). The quantification of amino acids was achieved by an external standard amino acid mixture of known concentrations (AAS-18, Sigma) supplemented with tryptophan, asparagine, cysteine, and glutamine. The limit of detection for amino acids are listed in Table 2.

On the other hand, for sugars analysis, liquid chromatography separations were carried out with XBridge Amide column 50 mm × 4.6 mm (Waters, Milford, MA, USA), and the elution was performed by applying isocratic elution for 30 minutes, Mobile phase was 70/30 acetonitrile/water with 0.1% ammonium hydroxide. The flow rate was 500 µL/minutes, and the mass spectrometer worked in the negative electrospray ionization (ESI) mode. We set spray voltage at 4.5 kV, and the ion transfer capillary temperature was 275°C. The masses of precursor and product ions and collision energy for each sugar are listed in Table 3. The data were processed as amino acids described above using an external standard mixture of

known concentrations. The limit of detection for quantification of sugars are listed in Table 4.

2.3 Statistical Analysis

The concentration of individual amino acids, the total concentration of free amino acids, the total concentration of essential amino acids, and the total concentration of individual sugars were all checked for normal distribution using the Kolmogorov-Smirnov test. One-way ANOVA was

used to analyze the data on the individual concentration of amino acids, total concentration of essential amino acids, and total concentration of sugars, while the total concentration of free amino acids was analyzed using the independent samples Mann-Whitney U-test. A statistical significance level of $P = 0.05$ was considered for all analyses, and the IBM SPSS Statistics Package V.26.0 (SPSS Inc., NY, USA) was utilized for the analyses.

Table 1. Precursor, product ions, and collision energy for amino acid analysis

Amino acid	Precursor ion $[M+H]^+(m/z)$	Product ion (m/z)	Collision energy (eV)
Glycine	76	30	50
Alanine	90	44	50
Serine	106	60	35
Proline	116	70	35
Valine	118	72	35
Threonine	120	74	35
Cysteine	122	105	35
Isoleucine	132	86	35
Leucine	132	86	35
Asparagine	133	87	35
Aspartic acid	134	116	35
Lysine	147	130	35
Glutamine	147	130	35
Glutamic acid	148	102	35
Methionine	150	104	35
Histidine	156	110	35
Phenylalanine	166	120	35
Arginine	175	158	35
Tyrosine	182	136	35
Tryptophan	205	188	35

Table 2. Limit of quantification (LOQ) for amino acids

Amino acid	Limit of Quantification (LOQ, μM)
Glycine	4.79
Alanine	1.99
Serine	0.35
Proline	0.11
Valine	0.03
Threonine	0.15
Cysteine	0.21
Isoleucine	0.08
Leucine	0.07
Asparagine	2.28
Aspartic acid	0.99
Lysine	0.17
Glutamine	0.34
Glutamic acid	0.67
Methionine	0.39
Histidine	0.29
Phenylalanine	0.05
Arginine	0.41
Tyrosine	0.79

Table 3. Precursor, product ions, and collision energy used for sugar analysis

Sugar	Precursor ion [M-H] ⁻ (m/z)	Product ion (m/z)	Collision energy (eV)
Fructose	179	143	35
Glucose	179	143	35
Sucrose	341	179	35
Trehalose	341	179	35
Melezitose	503	323	35

Table 4. Limit of quantification (LOQ) for sugars

Sugar	Limit of Quantification (LOQ, mg/L)
Fructose	0.80
Glucose	0.87
Sucrose	0.50

3. RESULTS AND DISCUSSION

3.1 Results

Eighteen amino acids were detected in both plants (Fig. 1a). Glycine and Cysteine were below detectable limits. The mean concentration of eight amino acids varied significantly between the two plants, arginine F (1, 17) = 6.315, P = 0.023, histidine F (1,17) = 40.484, P < 0.0001, lysine F (1,17) = 9.092, P = 0.008, proline F (1,17) = 10.324, P = 0.005, asparagine F (1,17) = 59.996, P < 0.0001, Glutamine F (1,17) = 8.312, P = 0.011, tyrosine F (1,17) = 14.429, P = 0.002 and

phenylalanine F (1,17) = 10.324, P = 0.005 all of these amino acids were present in higher concentration in broad bean than in alfalfa (P < 0.05). Moreover, in alfalfa, the most abundant amino acids were all non-essential amino acids, asparagine (36.03%), glutamic acid (17.37%) and Serine (11.92%). Similarly, in broad bean most abundant amino acids were non-essential amino acids, asparagine (60%), tyrosine (7.08%) and glutamic acid (3.91%) (Table 5). Total concentration of free amino acids in broad bean (Median: 86.3928, Q1:32.6571-Q3:150.4747 μMol/L) was significantly higher (U = 236, P = 0.019) than in alfalfa (Median = 13.12, Q1:6.2868-Q3:99.60 μMol/L) (Fig. 1b). Similarly, total concentration of essential amino acids in broad bean were higher than in alfalfa (P < 0.0001) (Fig. 1c). Three most abundant essential amino acids in alfalfa were Threonine (31.28%), Leucine (16.1%) and Valine (15.72%) while in broad bean the three most abundant essential amino acids were Histidine (48.4%), Lysine (14.04%) and Valine (9.70%) (Table 6).

Table 5. Total concentration (μMol/L) and percentage of amino acids from alfalfa and broad bean leaves, n= 9, different letters in the same row indicate significant difference at α= 0.05

Amino acids	Alfalfa		Broad bean	
	μMol/L	Percent (%)	μMol/L	Percent (%)
Arginine	8.82	0.86	110.82	2.86
Histidine	6.58	0.64	231.79	5.98
Lysine	5.40	0.53	67.21	1.73
Serine	122.39	11.92	150.12	3.87
Alanine	94.34	9.19	111.60	2.88
Proline	10.54	1.03	49.14	1.27
Asparagine	369.93	36.03	2316.64	60.00
glutamine	24.13	2.35	126.94	3.28
Threonine	30.99	3.02	33.08	0.85
Glutamic acid	178.34	17.37	151.53	3.91
Valine	15.58	1.52	46.45	1.20
Methionine	1.85	0.18	2.48	0.06
Aspartic acid	115.37	11.24	105.58	2.72
Tyrosine	3.85	0.37	274.47	7.08
Isoleucine	10.36	1.00	31.38	0.81
Leucine	15.95	1.55	18.76	0.48
Phenylalanine	10.66	1.04	43.25	1.12
Tryptophan	1.72	0.17	4.41	0.11
Total Conc.	1026.80 ^a		3875.64 ^b	

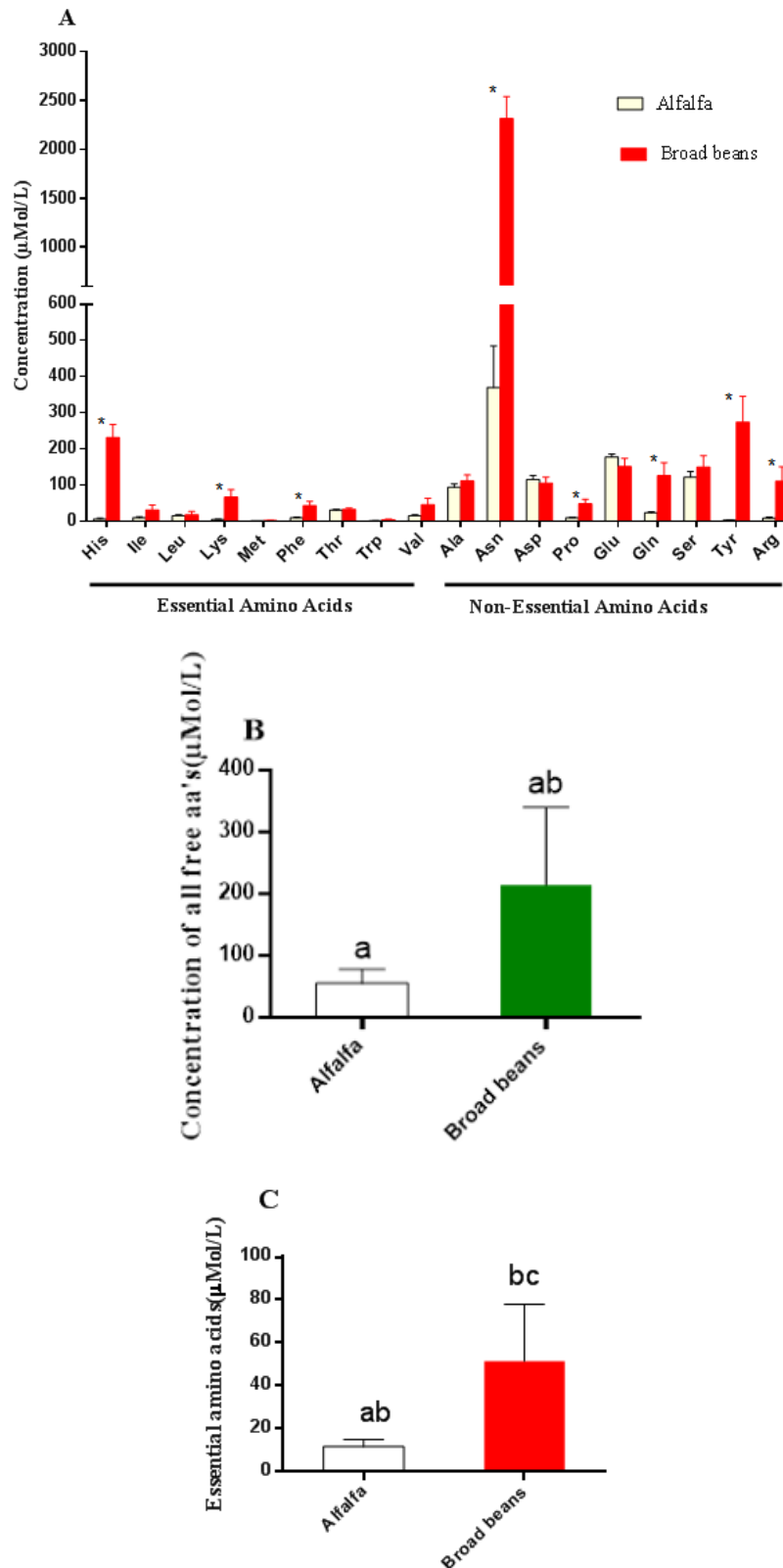


Fig. 1. Concentration of individual amino acids in alfalfa and broad bean (a), Concentration of total amino acids in alfalfa and broad bean (b), and the total concentration of essential amino acids in alfalfa and broad bean (c). The bars show Mean $\pm$ SE. Different letters and asterisks indicate $P < 0.05$

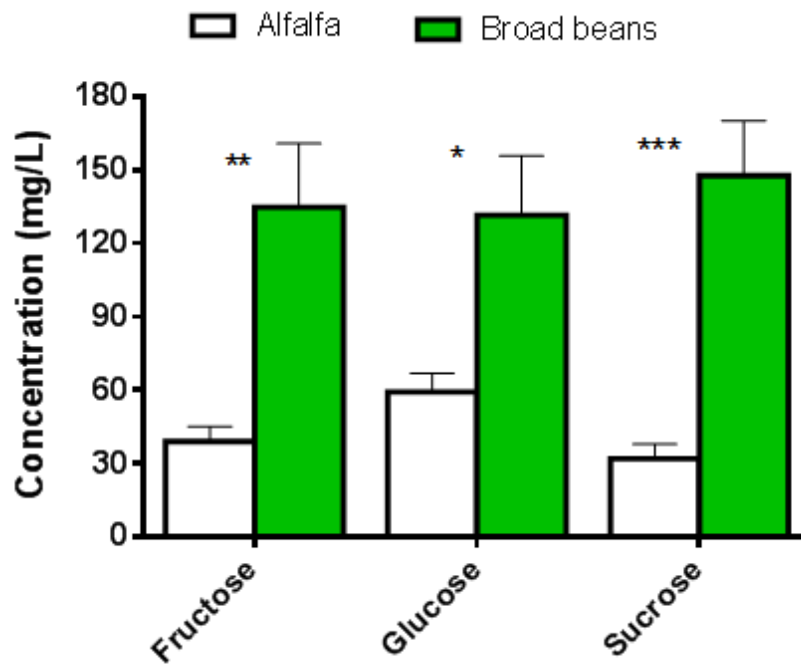


Fig. 2. Mean concentration of individual sugars in leaves of broad bean and alfalfa ($n = 9$)

Bars show mean $\pm$ SE. ***, $P < 0.001$; **, $P < 0.01$, and *, $P < 0.05$

Fructose, glucose, and sucrose were identified in the two plants. The total concentration of individual sugars varied significantly between the two plants (Table 7). However, sugars contained in the broad bean were all significantly higher than those in alfalfa, fructose $F(1,6) = 12.634$, $P = 0.003$, glucose $F(1,6) = 8.078$, $P = 0.012$ and sucrose $F(1,6) = 24.877$, $P < 0.0001$ (Fig. 2).

3.2 Discussion

Determinants of host plant quality by aphids are the allelochemicals, anatomical features, and nutrients (Agrawal, 2000; Liu et al., 2016). However, nutrients play a significant role as it constitutes of a wide range of elements such as amino acids, sugars, lipids, water, metals and other compounds useful for growth and development of the plant. Amino acids and sugar account for more than 90 percent of phloem sap dry matter (Cao et al., 2018). Generally, the analysis found that both broad beans and alfalfa contained a higher total amount of sugars than amino acids. Liu et al. (2016) also found that sugars were present in excess than amino acids in aphid diets. This could be because sugars are the available energy source and provide energy for the metabolic process associated with the plants and insects' humoral and cellular immune reactions (Hoffman et al., 2015; Mohammad et

al., 2012; Bala et al., 2018; Martin et al., 2023). The excess sugars in aphid diets are advantageous to aphids because these insects use excess sugars to detoxify toxic plant compounds, which normally pose a feeding hindrance (Malka et al., 2020).

Furthermore, results showed that broad beans contained a higher sucrose amount while alfalfa contained a higher glucose amount. This may be because natural diets of aphids contain higher sucrose concentrations (Ashford et al., 2000; Terra et al., 2005). The differences seen in the total amount of sugars have implications for insects' carbon nutrition and osmoregulation; see detail in Ashford et al. (2000). A low total amount of amino acids in broad beans and alfalfa was expected and are in accord with the available literature that plant phloem sap contains a lower concentration of total amino acids than sugars, and that is the limiting factor for aphid feeding (Ashford et al., 2000; Wilkinson et al., 2001; Awmack et al., 2002; Alaux et al., 2010; Dixit et al., 2013; Liu et al., 2016; Wilson et al., 2019).

In both plants, Asparagine was the most dominant amino acid than any other amino acid. This may be because of its storage and transport role of nitrogenous compounds in plants (Pate et al., 1981; Lea et al., 2007). These results are in

parallel with many past publications. Wilkinson et al. (2000) found that broad beans had a higher concentration of asparagine than any other amino acids. Correspondingly, Ta et al. (1986) found that asparagine was present in a higher amount in the nodules and root xylem exudates of alfalfa. Also, Scharff et al. (2003) reported that pea root nodules contained a high concentration total amount of asparagine. Similarly, in apple trees, asparagine was the dominating free amino acid (Malaguti et al., 2001). In white lupin (*Lupinus albus*), asparagine was the major amino acid in all plant parts, and it accounted for 60-80% of the total free amino acids quantified (Pate et al., 1981). Asparagine is the most dominating amino acids in leaves, seeds, and roots of plants (Sieciechowicz et al., 1988); it has a significant role in the storage and transport of nitrogen in many plants due to its N: C ratio of 2:4 (high) which makes it an efficient molecule for storage and transportation of Nitrogen in leguminous plants (Lea et al., 2007).

The second most abundant amino acid was tyrosine (Fig. 1a), and it was more abundant in broad beans than in alfalfa. Sandstrom et al. (1994) also found that tyrosine concentration was higher in broad beans than in alfalfa. Both insects and plants require tyrosine; in plants, it is a precursor for lignin synthesis and melanin in insects (Schenck et al., 2018). Results further showed that in alfalfa, the three most abundant amino acids were all non-essential amino acids, Asparagine (36.03%), Glutamic acid (17.37%), and serine (11.92%). Similarly, in broad beans,

the three most abundant amino acids were all non-essential amino acids, Asparagine (60%), tyrosine (7.08%), and glutamic acid (3.91%), so the two plants are predominantly comprised of non-essential amino acids. Similarly, Liu et al. (2016) and Liu et al. (2020) found that plant phloem saps are dominated by non-essential amino acids.

Also, results indicated that the total concentration of essential amino acids was high in broad beans than in alfalfa. Similarly, Sandstrom et al. (1994) found that the total concentration of essential amino acids was high in broad beans than in alfalfa. In this regard, broad beans are more nutritious than alfalfa because the nitrogen content of plant phloem sap, mainly the essential amino acids, is commonly used to index the plant's nutritional value for sap sucking insects (Liu et al., 2020). The low quality of alfalfa might be caused by Glutamic acid, which was higher in alfalfa than in broad beans (Liu et al., 2020).

The most dominant essential amino acid was histidine, which was also most abundant in broad bean. This amino acid cannot be synthesized *denovo* by animals, so they have to get it from plants. It is crucial for the growth and repair of the body cells. It functions as a chelator and transporter of metal ions (Stepansky et al., 2006). In higher plants, histidine is essential for plant growth and survival (Mo et al., 2006). It is necessary for protein synthesis and is required by all living organisms (Mo et al., 2006).

Table 6. Total concentration of free essential amino acids ($\mu\text{Mol/L}$) and percentage of each Eaa's in alfalfa and broad bean leaves. Different letters indicate a significant difference at $P < 0.05$ in the essential amino acids between alfalfa and broad bean leaf samples

Eaa's	Alfalfa		Broad bean	
	$\mu\text{Mol/L}$	Percent (%)	$\mu\text{Mol/L}$	Percent (%)
Histidine	6.58	6.64	231.79	48.41
Isoleucine	10.36	10.46	31.38	6.55
Leucine	15.95	16.10	18.76	3.92
Lysine	5.40	5.5	67.21	14.04
Methionine	1.85	1.87	2.48	0.52
Phenylalanine	10.66	10.76	43.25	9.03
Threonine	30.99	31.28	33.08	6.91
Tryptophan	1.72	1.74	4.41	0.92
Valine	15.58	15.72	46.45	9.70
Total Conc.	99.09 ^a		478.78 ^b	

Table 7. Total concentration of sugars (mg/L) from leaves of alfalfa and broad beans $n = 9$, different letters in the same row indicate significant difference at $\alpha = 0.05$

Sugar	Alfalfa		Broad bean	
	mg/L	Percent (%)	mg/L	Percent (%)
Fructose	39.08	29.97	134.60	32.54
Glucose	59.29	45.47	131.44	31.78
Sucrose	32.02	24.56	147.57	35.68
Total	130.39 ^{ab}		413.61 ^{bc}	

4. CONCLUSION

The analysis of amino acids and sugars in leaves of broad beans and alfalfa indicated that broad beans are more nutritious than alfalfa. Due to the low nutrients in alfalfa, aphids feeding on this plant might have poorer growth, survival, and reproduction compared to those feeding on more nutritious plants like broad beans. Therefore, broad bean is an ideal host plant for rearing pea aphids in the laboratory for research purposes. This information could be valuable for researchers working with pea aphids in controlled laboratory settings.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

ACKNOWLEDGEMENTS

This work was supported by the China Scholarship Council (CSC).

COMPETING INTERESTS

Author has declared that no competing interests exist.

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