Original Research Article

**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 (e.g., Pea aphids)**

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 the two nutrients in the leaf samples. The results showed that Vicia faba had higher concentrations of certain amino acids compared to Medicago 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

The leaves of broad beans and alfalfa are the main sites where pea aphids feed. Therefore, it is very important to measure the individual amino acids and sugars in the leaves to study how these two nutrients affect the physiology of the aphids. While there are other important nutritional factors for the aphids, amino acids and sugars are the main nutrients found in the phloem sap that are known to influence the aphids' physiology (Karley et al., 2002; Clissold et al., 2015; Nalam et al., 2019). Factors such as water, lipids, and secondary metabolites may influence phloem sap quality, but they are not as crucial as amino acids and sugars (Sandstrom et al., 1994; Sandstrom, 2000; Karley et al., 2002). The quality of phloem sap plays a significant role in determining the suitability of plants for aphids (Karley et al., 2002).

The phloem sap of plants contains high levels of sugars and low levels of amino acids (Sandstrom et al., 1994; Dixit et al., 2013; Nalam et al., 2019). Amino acids are crucial for the nutrition of pea aphids and are strongly linked to their population growth, survival, and reproductive success (Sandstrom et al., 1994; Weibull et al., 1990; Awmack et al., 2002). Plants with lower concentrations of total amino acids are more resistant to aphids compared to those with higher amino acid concentrations (Maltais et al., 1957). Stressed plants have altered nutritional quality and improved defense against aphids (Xie et al., 2020). The nutritional quality of plants plays a significant role in plant-insect interactions (Cao et al., 2016). Therefore, it is important to analyze the amounts of sugars and amino acids in the plants used to rear the test insects. This study investigated the amount of amino acids and sugars in broad beans and alfalfa leaves. Although other plant qualities, such as secondary metabolites, are also present, sap-sucking insects like pea aphids have developed mechanisms to tolerate and store these secondary metabolites for protection against various threats. These metabolites do not therefore significantly hinder aphid feeding on phloem sap compared to amino acids and sugars. Sugars and amino acids make up about 90% of the total dry matter in phloem sap and are the primary nutritional factors for the aphid diet (Cao et al., 2016).

2. material 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), Paracios et al. (2015), 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.

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-Q3:150.4747µMol/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).

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 F1,6= 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 et al., 2001; 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). 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 (Osnat 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; Dexit 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).

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.

Consent

Not applicable

Ethical approval

Not applicable

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APPENDIX

 **LIST OF TABLES & FIGURES**

**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 |

**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

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  Alfalfa |  |  Broad bean |  |
| Amino acids | 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. | 1026.80a |  |  3875.64b |  |

**Table 6**: Total concentration of free essential amino acids (µMol/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.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  Alfalfa |  | Broad bean |  |
| Eaa’s | **µMol/L** | **Percent (%)** | **µMol/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. | 99.09a |  | 478.78b |  |

**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 α= 0.05

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Alfalfa |  | Broad bean |  |
| Sugar | 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 | 130.39ab |  | 413.61bc |  |

|  |
| --- |
|   |

**Figure 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±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 ±SE. \*\*\*, *P* < 0.001; \*\*, *P* < 0.01, and \*, *P* < 0.05.