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Influence of selected soil parameters on amino acid profile in *Stellaria media*

[1]Magdalena M. Dziągwa-Becker, [1]Olga Kalitowska, [2]Wiesław A. Oleszek

[1]Institute of Soil Science and Plant Cultivation – State Research Institute, Department of Weed Science and Tillage Systems, ul. Orzechowa 61, 50-540, Wrocław, Poland
[2]Institute of Soil Science and Plant Cultivation, State Research Institute, Department of Biochemistry and Crop Quality, Puławy, Poland

Abstract. The study investigates the relationship between soils that vary for different basic physicochemical parameters (pH, phosphorus and potassium content, granulometric composition and soil organic matter content) and the free amino acids content of *Stellaria media*. According to some research, plant amino acid profile is a good indicator of the condition of the soils on which plants are grown. In this experiment, a rapid and sensitive liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) method was used for the identification and quantification of nineteen proteinogenic amino acids in *Stellaria media* samples, which is a very common weed found worldwide. Significant linkage was found between the soil parameters and *Stellaria media* amino acid content. Garden soil is the most favourable soil for amino acid content. Glutamine, glutamic and aspartic acid are the most abundant amino acids in *Stellaria media* grown on all three tested soils.

Keywords: free amino acids, LC-MS/MS, soil organic matter, soil quality, common chickweed

INTRODUCTION

Soil is a vital component of biosphere and crucial reservoir of nutrients for plants. The study of soil physicochemical parameters is important in terms of plant growth requirements and soil management (Osman, 2013). Changes in the amount of some plant metabolites indicate to what extent their functions are induced or repressed (Last et al., 2007). Amino acids (AA) are reported to be primary metabolites, playing central roles both as building blocks of proteins and as intermediates in metabolism. Twenty three AA are regarded as proteinogenic, meaning that they are precursors to proteins (Berg et al., 2002). Resistance to stress in plants may, to some extent, be determined by analysing amino acid metabolism, in which osmotic adjustment and the accumulation of compatible osmolytes, detoxification of active oxygen species and risk element play a special role (Last et al., 2007). There is, at present, scanty information on the amino acid profile of plants, therefore very common weed – *Stellaria media* was chosen as a bioindicator of various soils in the presented research, due to its cosmopolitan character and low habitat requirements (Sobey, 1987). The aim of this work was to investigate how basic soil parameters like pH, phosphorus and potassium content, granulometric composition and soil organic matter content, affect plant proteinogenic amino acids content.

Corresponding author:
Magdalena Dziągwa-Becker
m.dziagwa@iung.wroclaw.pl
tel. 666-012-444

MATERIAL AND METHODS

Plant material and soil specification

First, we collected *Stellaria media* seeds from plants grown in Zagródki. Secondly, we used these seeds to obtain unified material which we cultivated in a greenhouse. The self-obtained seeds were cultivated in a growth chamber under controlled conditions maintained at 21 °C (day), 15 °C (night), 14 hours of light and the soil moisture was kept at 60% of maximum water holding capacity (MWHC). Four different kind of soils were chosen for this experiment. A summary of soil physicochemical properties of the tested soils are shown in Table 1. The aboveground biomass was obtained just before flowering, both of stems and leaves.

Sample preparation

In order to determine AA content in plant samples, leaves and stems (0.5 g) were placed in a mortar and ground with a pestle using liquid nitrogen. The samples were extracted with LC-MS grade water, followed by 15 min sonification in ultrasonic bath. Homogenates were centrifuged at 11000 rpm for 15 min at 4ºC to obtain supernatants, ready to be analysed on the EZ:faast$^{(TM)}$ Free Amino Acid kit (Phenomenex, Torrance, CA, USA). The

Table 1. Physicochemical properties of the tested soils.

Soil	Localisation	pH 1 M KCl	SOM [%]	P_2O_5 [mg (100 g soil)$^{-1}$]	K_2O [mg (100 g soil)$^{-1}$]	Texture of soils [%]			Texture classes Polish Soil Society (2008)
						2000–50 µm	50–2 µm	<2 µm	
A	Zagródki	6.9	2.9	24.6	18.5	64	17	19	sandy loam
B	garden soil	6.6	>20	27.0	50.0	2:1 sphagnum peat:sand			
C	Laskowice	4.6	0.46	14.0	27.0	72	25	3	sandy loam
D	Wojsław	7.3	4.8	26.5	34.0	50	37	13	loam

procedure of using EZ:faast$^{(TM)}$ kit, which is transparent and straightforward (Badawy et al., 2008; EZ:faast$^{(TM)}$ User's Manual 2013), was performed according to the optimized and validated method presented by Dziągwa-Becker et al. (2015). Briefly, the procedure consists of solid phase extraction where plant extract was passed through the sorbent tip that bound amino acids, derivatization using propyl chloroformate, and liquid-liquid extraction step. Triple Quadrupole Mass Spectrometry is the method of choice for accurate identification and quantification of trace level analytes in complex matrices (Meesters et al., 2009).

Determination of free amino acids by high performance liquid chromatography with electrospray tandem mass spectrometry (LC-MS/MS)

Analysis was carried out using a high performance liquid chromatograph Shimadzu 8030 (Shimadzu, Kyoto, Japan) with a binary solvent manager, autosampler and column oven. For the chromatographic separation, an EZ:faast$^{(TM)}$4u AAA-MS column, 3 µm, 250 × 2.0 mm (Phenomenex, Torrance, CA, USA) at a flow rate of 0.25 mL min^{-1} was used. The column was kept at 35°C. Mobile phase consisted of water/methanol (A/B) gradient both 10 mM ammonium formate where the methanol percentage was changed linearly as follows: 0 min, 68%; 13 min, 83%; 13.01 min, 68%; 18 min, 68%. All the samples were analysed under the abovementioned chromatographic conditions. The sample volume injected in the HPLC system was 10 µL. The tandem mass spectrometer LCMS-8030 (Shimadzu, Kyoto, Japan) with ultra fast polarity switching and ultra fast MRM transitions was used for analysis (see Table 2). Drying gas as well as nebulising gas was nitrogen, obtained from pressurized air in a N_2 LC-MS pump, working at a flow rate 15 L min^{-1} and 3 L min^{-1}, respectively. Desolvation line temperature was maintained at 250 °C and heat block temperature was 400 °C. Collision-induced dissociation gas (CID) was argon 99.999% (Linde, Wrocław, Poland) at a pressure of 230 kPa. Dwell time of 10 ms was selected. For HPLC analysis, LabSolution Ver.

Table 2. Amino acids, AA abbreviated name, retention time, quantification and confirmation transitions chosen for each compound.

Compound name	Abbreviated name	t_R [min]	Quantification transition	Confirmation transition
Arginine	ARG	3.3	303.20>70.10	303.20>156.05 303.20>114.05
Glutamine	GLN	3.8	275.20>172.00	275.20>84.00
Serine	SER	4.4	234.20>146.00	234.20>104.00
Asparagine	ASN	4.4	243.20>157.20	243.20>115.10 243.20>211.30
Glycine	GLY	4.9	204.20>76.00	204.20>102.00 204.20>144.10
Threonine	THR	5.1	248.20>74.05	248.20>160.00 248.20>188.10
Alanine	ALA	6.1	218.20>130.20	-
Methionine	MET	8.1	278.20>190.15	278.20>142.00 278.20>218.00
Proline	PRO	8.2	244.20>156.05	244.20>70.20 244.20>113.95
Lysine	LYS	8.9	361.30>170.10	361.30>301.05 361.30>128.10
Aspartic acid	ASP	8.8	304.00>216.15	-
Histidine	HIS	8.9	369.90>110.15	369.90>196.15 369.90>284.20
Valine	VAL	9.2	246.20>158.15	246.20>116.05 246.20>186.05
Glutamic acid	GLU	9.4	318.20>230.05	318.20>258.10
Tryptophan	TRP	9.7	333.20>245.15	333.20>159.20 333.20>230.00
Leucine	LEU	10.9	260.20>172.15	-
Phenylalanine	PHE	10.9	294.20>206.20	294.20>120.05 294.20>163.95
Isoleucine	ILE	11.3	260.20>130.10	-
Tyrosine	TYR	13.3	396.20>136.05	396.20>222.00 396.20>308.10

- the second and third transition were not monitored for this compound

5.6 (Shimadzu, Kyoto, Japan) software was used to process quantitative data obtained from calibration standards and from plant samples. The samples were injected by triplicate, preceded by calibration curve. All the solvents used were of LC-MS grade and obtained from Fluka Analytical (St. Louis, MO, USA). The detection limit (LOD) for the free amino acids varied from 0.4 to 9.1 pmol mL^{-1}, except for asparagine amounting to 3000 pmol mL^{-1}. In the study, plant samples were analysed using liquid chromatography coupled to triple quadrupole tandem mass spectrometry.

Method validation

The method meets all the criteria for LC-MS/MS analysis found in SANCO/12571/2013 guidelines as well as ICHQ2(R1), which is method linearity, a square correlation coefficient (r^2) $\geq$ 0.99 with residues always lower than 20%, precision (expressed as repeatability in terms of relative standard deviation) measured both intra and interday lower than 2,5%, recovery expressed as accuracy between 70 and 120% and ion intensity ratio tolerance lower than 10%, which is in accordance with the maximum permitted tolerances using MS techniques (Dziągwa-Becker et al., 2015).

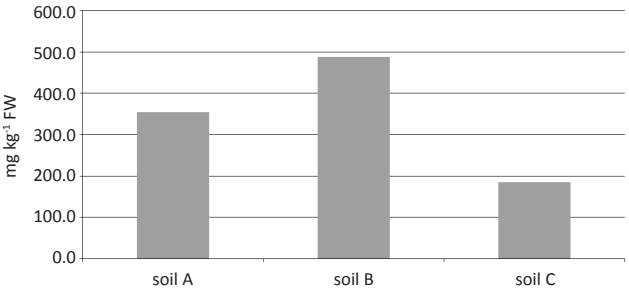
Soil properties determination

Basic physicochemical soil parameters like pH, phosphorus and potassium content, granulometric composition of soil and soil organic matter (SOM) content were determined for the three tested soils (A, C, D). Physicochemical properties of garden soil (soil B) were determined by the manufacturer. Soil pH was tested using 1 M KCl and measured potentiometrically, while potassium and phosphorus contents were tested according Egner-Riehm method. Determination of soil organic matter (SOM) was performed on the basis of C_{org} content, quantified according to the Tiurin's method. Granulometric composition was done using Casagrande method in Prószyński modification (Drozd et al., 1998; Kabała, Karczewska, 2008).

RESULTS AND DISCUSSION

Of the four soils under tesing soil D turned out to be innapropriate for *Stellaria media* growth and therefore nearly no biomass was produced. This could be explained when the pH value (7.3 in 1 M KCl – basic) is taken into account, because common chickweed prefers neutral soils to grow, although can be found on a broad range of pH (from 4.8 to 7.3). It seems that the border value is not favourable for the plant, even though the latter is known for great adaptability. All other analysed parameters, like potassium and phosphorus content or soil texture give irrefutable evidence to state that this soil has capability for plant cultivation indeed.

In the analysed plant samples all 19 proteinogenic amino acids were found, however their content varied significantly (see Figure 1 and 2). Free amino acids were the most abundant in *Stellaria media* grown on soil B, totaling 487.3 mg kg^{-1}. It may be explained in two ways. This soil has the highest soil organic matter (SOM) content, known to act as a plant nutrients reserve, especially carbon (C), nitrogen (N), phosphorus (P) and sulphur (S), first and second of them being a fundamental element building amino acids (Troeh, Thompson, 1957. Plants obtain carbon and oxygen from air while nitrogen is collected from soil. Moreover, the pH amounting 6,6 is neutral, which all together created the most beneficial conditions for the selected weed. A chromatogram obtained from *Stellaria media* cultivated on soil B can be seen on Figure 3.

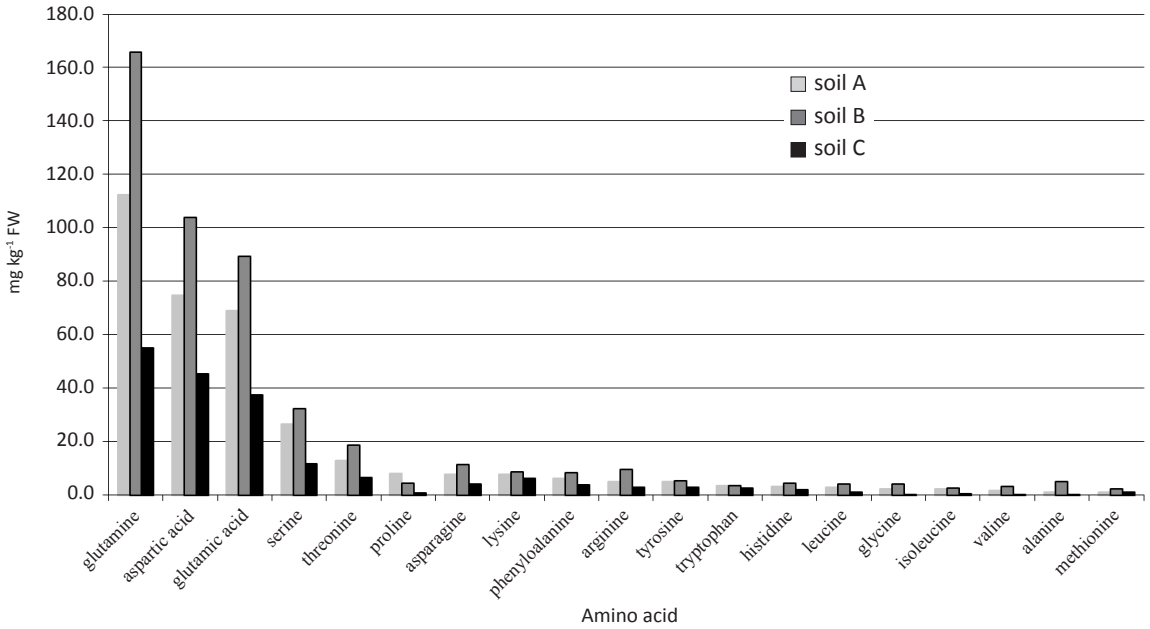


Physicochemical properties of the tested soils were presented in the Table 1

Figure 1. Total amino acid content of *Stellaria media* on different soils.

The second best result was obtained for soil A (354 mg kg^{-1}) where the SOM content amounts 2.9%. There was a noticeable decrease in the free amino acids content in comparison to soil B, although the results were much better than for the soil C.

When the differences between the individual amino acid are taken into consideration, some tendency can be observed. Glutamine on soil B reached the highest level among all other amino acids totaling 166 mg kg^{-1}. Its level is also the highest on soil A and C (112 and 55 mg kg^{-1}, respectively). This is in line with the results presented by Arnáiz et al. (2012) who also observed the highest amounts of glutamine and proline in broccoli leaves using supercritical fluid extraction. Glutamine and glutamic acid with proline, histidine, arginine and ornithine constitute the "glutamate family" of amino acids. Glutamine via glutamic acid is converted to alpha-ketoglutarate, an integral component of the citric acid cycle. It is a component of the antioxidant glutathione. L-Glutamic acid is a ubiquitous amino acid present in many foods either in free form or in peptides and proteins. Animal protein may contain from 11 to 22% and plants protein as much as 40% glutamate by weight. Interestingly, it is known to be a direct precursor to chlorophyll and heme biosynthesis, leading to higher degree of photosynthesis (Tapiero et al., 2002). Our



Physicochemical properties of the tested soils were presented in the Table 1.

Figure 2. *Stellaria media* amino acids content on different soils.

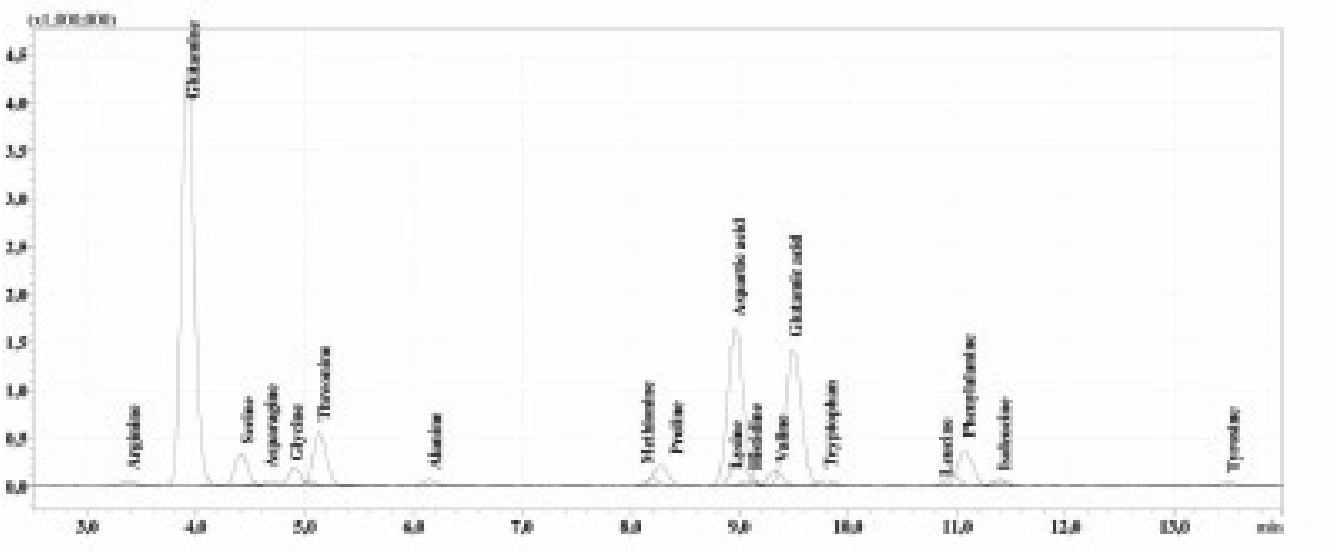


Figure 3. A chromatogram obtained from *Stellaria media* cultivated on soil B.

experiment shows that glutamic acid concentration is also significant in common chickweed; the third best result for this amino acid was found on all of the tested soils.

Furthermore, it was found that aspartic acid was the second most abundant amino acids on all of the tested soils, which is a common precursor of the essential amino acids lysine, threonine, methionine and isoleucine. These amino acids are not synthesised by human and should be supplemented in their diets (Galili, 2011).

Finally, we present results referring to proline level. This is a very distinctive amino acid and therefore is discussed separately. It is unique among the 23 proteinogenic amino acids, because the amine nitrogen is bound to not one but two alkyl groups, thus making it a secondary amine. Proline is known to accumulate in many plant species in response to environmental stress (Nazar et al., 2015; Rejeb et al., 2014; Szabados, Savourè, 2010; Misra, Saxena, 2009; Kovacs et al., 2012). While analysing pro-

line content, we can observe that on neither soil was its concentration significant (8.0, 4.3, 0.8 mg kg^{-1}; soil A, B and C, accordingly). In comparison to glutamine or aspartic acid concentration reaching 165.7 and 103.9 mg kg^{-1}, respectively on soil B, we can presumably conclude that even though the environmental conditions on some of the tested soils were not optimal for plant growth, the plant did not suffer due to stress. According to Rhodes and Handa (1989), when cell cultures of tobacco were adapted to 428 mM NaCl, proline represented over 80% of the free amino acid pool.

This experiment demonstrated how different soil parameters can affect free amino acids content in plants. The applied LC-MS/MS method is very sensitive, thus it is possible to detect amino acids at pmol level. Moreover, due to applied multiple reaction monitoring mode (MRM), individual amino acids are selectively confirmed and quantified. This is the first attempt to show influence of soil parameters on the free amino acids content in *Stellaria media*. Dziągwa-Becker et al. (2015) have analysed *Stellaria media* samples obtained from field locations and reported occurrence of nineteen free amino acids, where similarly glutamine was found in the highest level.

The very first conclusion that can be drawn from the experiment is that plant amino acid content rise together with the fertility of the soil on which they are grown, although the growth rate is not identical for all AA. A good indicator of this trait is SOM content. The pathways for the biosynthesis of amino acids are very diverse, although there is one common feature – their carbon skeletons come from intermediates of glycolysis, the pentose phosphate pathway or the citric acid cycle (Berg et al., 2002). Carbon improves physical properties of the soil. It increases the cation-exchange capacity (CEC) and water-holding capacity of sandy soil, and contributes to the structural stability of clay soils by helping to bind particles into aggregates. SOM holds a great reservoir of nutrients and trace elements that are of primary importance to plant growth. Carbon prevents nutrient leaching and constitutes an integral part of organic acids that make minerals available to plants. It also buffers soil from strong changes in pH (Zawadzki, 1995).

Soil C which was classified as sandy loam, achieved the poorest results for amino acids. It belongs to the third soil category according to IUNG-PIB, Puławy (Kabata-Pendias et al., 1993). Its fertility and applicability is weather-dependent. This soil is poorly-abundant in SOM (only 0.46%) with medium phosphorus and very high potassium content, which is due to mineral fertilization. Soil A is also sandy loam, belonging to the third soil category but its texture is rather different from soil C. It seems that on soil A, the primary factor determining its quite high amino acid content was optimal (neutral) pH for *Stellaria media* and 2,9% of SOM. This soil also has very high phosphorus and medium potassium content.

To summarise, amino acids in plants play numerous different functions from being building blocks of proteins, nitrogen carriers in transport systems, precursors to important metabolites, nitrogen storage molecules, to being stress response and signaling molecules (Okumoto et al., 2016). These various AA functions, together with dynamic metabolite flux in plants and diverse environmental conditions make this kind of experiments rather challenging.

CONCLUSIONS

1. Free amino acid content is interrelated with soil properties, mostly with SOM content and soil pH.
2. Free amino acid content of *Stellaria media* rises together with the fertility of the soil latter expressed as SOM.
3. The chromatographic method to determine free amino acids in plants applied in this study is very sensitive and robust. Glutamine is the most abundant amino acid in *Stellaria media* grown on all three tested soils.

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