

Original Research Article

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## Foliar Amino Acids Sprays on Lettuce (*Lactuca sativa* L.) Biomass Accumulation

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### ABSTRACT

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Nitrogen has been associated to lettuce fresh weight accumulation. In this way, different bio stimulants formulations containing amino acids have been commercially offered although the yield increase has not been properly supported. The aim of this work was a) to quantify the changes in lettuce fresh weight accumulation and the physiological mechanism involved in plants sprayed with different amino acid solutions and (b) to characterize the responses to root restrictions under nursery with non-limited nitrogen supply. Our results showed that a foliar spray with different amino acids each alone or combined can change positively or negatively lettuce fresh-dry weight, total leaf area and photo assimilate partitioning under a nursery abiotic stress related to plug cell size. Although, exogenous amino acids supply can be suggested as signalling molecules, the presence of limited plug cell volume (288-plug cell tray) would be demanded for the use of more than a single one. On the other hand, although additive and antagonistic effects have been suggested, conclusive results needs for further experiments.

### Introduction

Both availability and uptake of nitrogen are considered as the major factors affecting plant growth. Nitrogen acquisition by plant roots occurs to a variety of inorganic (nitrate, ammonium and urea) and organic (amino acids and peptides) nitrogen sources (Andrews *et al.*, 2013). Nitrogen metabolism exerts a strong effects on both photo assimilates fixation and partition between roots and shoots (Poorter *et al.*, 2012). Nitrate reductase, nitrite

reductases and the glutamate synthase cycle catalyzed organic nitrogen assimilation. The Glutamate occupies a central position in amino acid metabolism in plants (Filleur *et al.*, 2005; Schachtman and Shin, 2007).

Main amino acids (glutamate) have been correlated with nitrogen acquisition while minor amino acids such as arginine, phenylalanine, tyrosine and valine have been correlated with photo assimilates storage (Foyer *et al.*, 2003). At the same time,

arginine and proline have been indicated as precursors of polyamides associated to the plant response towards abiotic stresses (Forde and Lea, 2007; Biancucci *et al.*, 2015) and functioned as signaling molecules (Häusler *et al.*, 2014).

On the other hand, Stitt *et al.*, (2002) showed that addition of ammonium, glutamine, or glutamate, did not lead to a significant increase in the minor amino acids levels, indicating that flux into the amino acid biosynthesis pathways is not limited by the availability of glutamate in main plant metabolism, at least in plants where the nitrogen supplies is not limiting.

Previous reports indicate that exogenous amino acids supply increased growth and yield to different horticultural species such as lettuce (Polo *et al.*, 2006), celery (Shehata *et al.*, 2011), spinach (Kunicki *et al.*, 2010), leaf radish (Liu *et al.*, 2008), pepper (Haghighi and Barzegar, 2017) and squash (El-Aal *et al.*, 2010) but, the precise physiological mechanism involved in these responses remained unsolved.

Lettuce is a major crop grown worldwide (Stagnari *et al.*, 2015; Shehata *et al.*, 2016). Lettuce yields increase according to photosynthetic light increase (He *et al.*, 2011) as a response to a nitrate reductase increase (Stagnari *et al.*, 2015). In the same way, lettuce yields are positively related to nitrogen supply (Linker and Johnson-Rutzke, 2005). However, lettuce is also prone to accumulation of nitrates, which are recognized to be dangerous to human health (Fewtrell, 2004).

Transplanting has been thoroughly replacing vegetables direct seeded since plug trays have been commercially offered in spite of the root restriction syndrome related to the limited plug cell volume (Araki *et al.*, 2007; Di

Benedetto, 2011; Coro *et al.*, 2014; Sanchez Andonova *et al.*, 2014; Di Matteo *et al.*, 2015).

The objective of this work was to analyze lettuce growth changes in the presence of different root restrictions degree by the use of different plug cell volumes but sprayed with amino acids solutions under the hypothesis that they would play a role as abiotic stress alleviators.

## Materials and Methods

The experiment was carried out in the Faculty of Agronomy campus, University of Buenos Aires, Argentina (34°35' 59''S, 58°22' 23''W) between October 30<sup>th</sup> 2017 and February 26<sup>th</sup> 2018.

To reach proposed objectives, lettuce (*Lactuca sativa* L.) Crimor INTA seeds were grown in 50- (55.7cm<sup>3</sup> cell<sup>-1</sup>) and 288- (6.18 cm<sup>3</sup> cell<sup>-1</sup>) plug trays in a Klasmann411® medium (Klasmann-Deilmann, GmbH, Germany) for 35 days. When seedlings reached the transplant stage, they were transplanted into 3 liters pots filled with a *Sphagnum maguellanicum*-river waste-perlite (40-40-20, v/v/v) medium. At the beginning of the experiments total porosity (%), air-filled porosity (%), container capacity (%) and bulk density (g cm<sup>-3</sup>) were 63.50, 17.06, 10.06 and 0.35 respectively. Weeds were manually removed.

Eleventh solutions (100 mg L<sup>-1</sup>) containing an equal amino acids number (glutamic acid, aspartic acid, alanine, cysteine, glycine, leucine, lysine, methionine, threonine, tryptophan and valine), a control without treatment and a solution containing all amino acids (Mix) (100 mg L<sup>-1</sup>) were tested. Leaves were sprayed at sunset when the first true leaf pair was developed. Plants were irrigated as needed with high quality tap water (pH: 6.64

and electrical conductivity of 0.486 dS m<sup>-1</sup>) using intermittent overhead mist to compensate evapotranspiration losses. Growing media was weekly fertilized with 1:1:1:1 (v/v/v/v) N: P: K: Ca through the overhead water irrigation (Stage 2: 50 mg L<sup>-1</sup> N; Stage 3-4: 100 mg L<sup>-1</sup> N; pot: 200 mg L<sup>-1</sup> N) was used.

Daily mean temperatures (18.05 to 24.86 °C) and daily photosynthetic active radiation (6.83 to 11.16 mole photons m<sup>-2</sup> day<sup>-1</sup>) for the experiment were recorded with a HOBO sensor (H08-004-02) (Onset Computer Corporation, MA, USA) connected to a HOBO H8 data logger. The plants were arranged at a density of six plants m<sup>-2</sup>, which avoided mutual shading.

Plants were harvested at the transplant stage and at 20, 40, 60, 80, 100 and 120 days from transplanting. Roots were washed and root, stem and leaf fresh weights (FW) were recorded. Dry weights (DW) were obtained after drying roots, stems and leaves to constant weight at 80°C for 96 hours. The number of leaves was recorded, and each leaf area was determined using the ImageJ® (Image Processing and Analysis in Java) software.

The relative rate of leaf area expansion (RLAE) was calculated as the slope of the regression of the natural logarithm (ln) of total leaf area versus time (in days). The rate of leaf appearance (RLA) was calculated as the slope of the number of fully expanded leaves versus time (in weeks). The relative growth rate (RGR) was calculated as the slope of the regression of the natural logarithm (ln) of whole plant DW versus time (in days).

Mean net assimilation rate (NAR) was calculated as follows:

$$NAR = \frac{k_w W_0 e^{k_w t}}{A_0 e^{k_a t}}$$

where W<sub>0</sub>: extrapolated value of total DW (g) at time zero; k<sub>w</sub>: RGR (g g<sup>-1</sup> day<sup>-1</sup>); A<sub>0</sub>: extrapolated value of leaf area (cm<sup>2</sup>) at time zero; k<sub>a</sub>: RLAE (cm cm<sup>-2</sup> day<sup>-1</sup>); t: time (days) at the midpoint of the experimental period and e: base of the ln.

Mean and leaf area ratio (LAR) was calculated as follows:

$$LAR = \frac{k_w}{NAR}$$

The specific leaf area (SLA) and the leaf weight ratio (LWR) on a dry weight (DW) basis were calculated as the ratio of leaf area: leaf DW and the ratio of leaf DW: total DW respectively between the transplant stage and the end of the experiment.

The allometric coefficients between root and shoot were calculated as the slope (β) of the straight-line regression of the ln of the root DW versus the ln of the shoot DW (ln root DW = a + b x ln shoot DW). The Root: Shoot ratio (at the end of the experiment) was performed as well.

We used a complete aleatory design. Data were subjected to one-way analysis of variance and means were separated by Tukey's test (P < 0.05); STATISTICA 8 (StatSoft) software was used. Least significant differences (LSD) values were calculated. Slopes from straight-line regressions of RLAE, RLA, RGR and allometric value were tested using the SMATR package (Warton *et al.*, 2012).

## Results and Discussion

Significant statistical differences in post-transplant fresh weight accumulation have been found in lettuce plants sprayed with 12 amino acids solutions during nursery. Plants grown in 50-cell tray<sup>-1</sup> showed higher values than those

from 288-cell tray<sup>-1</sup>. At the end of the experiment (120 days from sowing) 50-cell plug tray<sup>-1</sup> plants showed no significant increases in most of the amino acids solutions sprayed and controls but, significant decreases in fresh weight in some amino acids solutions tested (Mix, glutamic acid, cysteine, glycine, leucine, valine). On the other hand, lettuce seedlings grown in 288-cell plug tray<sup>-1</sup> showed a significant increase related to controls when a combined solution (Mix) was applied and higher response heterogeneity between the rest single-applied amino acids (Table 1).

Under a relatively low plug cell volume abiotic stress, this is, in plants grown in 50-cell plug tray<sup>-1</sup>, total leaf area differences between controls and amino acids ones were significant in the latter two harvest time (data not shown). In plants grown at 50-cell plug tray<sup>-1</sup>, the higher total leaf area was found in alanine-sprayed plants; on the contrary, plants sprayed with aspartic acid, glycine and leucine showed the lowest values even to control plants. However, when seedlings were grown in 288-cell plug trays during nursery, the higher total leaf area was found in the Mix solution following by control plants (Table 2).

The higher control RLAE and RLA values were found in plants from 50-cells plug tray<sup>-1</sup>, while an inverse SLA results was found. The Mix solution which contained the thirteen amino acids tested, increased RLAE and RLA in plants grown in 288-cells plug tray<sup>-1</sup>. In 50-cells plants, all amino acids sprayed increased RLAE on control and Mix plants, although showed a heterogenic response from RLA (Table 2).

Plants grown in 50-cells plug tray<sup>-1</sup> and sprayed with glycine and leucine, showed the lower shoot dry matter accumulation while the lower root dry weight values was found in control plants (Figure 1A). Plants grown in 288-cells plug tray<sup>-1</sup>, the higher shoot dry weight was

found in the Mix, leucine, lysine, threonine and tryptophan sprayed plants. Once again, the lowest root dry weight was found in control plants (Figure 1B). Anyway, plants from 50-cells plug tray<sup>-1</sup> showed even the highest both shoot and root dry weight than those from 288-cells plug tray<sup>-1</sup>.

The higher both RGR and NAR were found in plants from 50-cells than in those from 288-cell plug tray<sup>-1</sup> while an inverse LAR response was found. RGR did not change significantly from 50-cells grown plants amino acid-sprayed while the Mix treatment increased RGR in 288-cells grown plants. All of the twelve amino acids solutions increased 50-cell grown plants NAR but only the Mix treatment increased 288-cell grown plants NAR. LAR showed only significant minor changes from 50-cell plug tray<sup>-1</sup> and no significant differences in those from 288-cell plug tray<sup>-1</sup> (Table 3).

When NAR and LAR values were plotted together, positive relationships between NAR, LAR and RGR were found (Figure 2). Lettuce plants from 50-cell plug tray<sup>-1</sup> showed higher determination coefficients ( $r^2$ ) than those from 288-cell plug trays.

Higher root: shoot ratios were found in plants from 50-cells plug tray<sup>-1</sup> than those from 288-cells plug tray<sup>-1</sup>. Amino acids-sprayed plants from 50-cell plug tray<sup>-1</sup> increased root: shoot ratio, except for the Mix and tryptophan-sprayed ones. On the other hand, in plants from 288-cell plug tray<sup>-1</sup> all amino acids treatments increased root: shoot ratio over controls. A similar trend was found when the root: shoot allometries were performed. The higher partition coefficient ( $\beta$ ) was found in plants from 288-cell plug tray<sup>-1</sup> and in control plants from both cell volume treatment (Table 4).

Despite the technological use of plug cell trays in vegetable industry, they imply the presence of an abiotic root restriction stress (Di

Benedetto, 2011). To override these effects on plant growth, the use of different bio stimulants commercial formulations has been proposed (Rai, 2002; Shehata *et al.*, 2011, 2016; Colla *et al.*, 2015; Häusler *et al.*, 2014). However, the most physiological mechanism involved has not been completely documented. Biostimulant meaning included all substance or microorganism exogenously supplied to plants which increased growth, environmental stress tolerance or traits related with the commercial quality (Du Jardin, 2015). In this context, the use of amino acids would be justified, although a few data on vegetables are available (Polo *et al.*, 2006; Liu *et al.*, 2008; El-Aal *et al.*, 2010; Haghghi and Barzegar, 2017; Kunicki *et al.*, 2010; Shehata *et al.*, 2011).

Our results showed a higher fresh weight accumulation at the end of the experiment in plants from 50-cells plug trays<sup>-1</sup> which suggested a lower root restriction level (Table 1) in agreement with previous reports (Williams *et al.*, 2016; Rattin *et al.*, 2017; De Lojo *et al.*, 2017; Geraci *et al.*, 2018). A foliar spray containing all the amino acids tested (Mix solution) when lettuce plants only had expanded the second true leaves pair, decreased fresh weight 120 days later. These results would be explained by the antagonistic effects showed by single amino acids solutions (glutamic acid, cysteine, glycine, leucine and valine) (Table 1). On the contrary, when plants from 288-cells plug trays<sup>-1</sup> were early sprayed with Mix solution, the higher fresh weight accumulation (similar to the controls 50-cells plug trays<sup>-1</sup>) was found although with significant differences between single amino acids solutions (Table 1), in agreement with Linker and Johnson-Rutzke (2005) and Mondal *et al.*, (2015).

At plant level, biomass accumulation on a fresh-dry weight is related to total leaf area expanded and photo assimilates both fixation

and partitioning (Andrews *et al.*, 2013). The effect of a root restriction related to plug cell volume on lettuce was similar to previously finding on fresh weight (Table 2), although significant differences between single amino acids solutions and controls lettuce increased as well.

Total leaf area is the result of leaf initiation and leaf expansion, estimated through RLA and RLAE respectively (Table 2). Although there is no previous report on the effect of amino acids sprays on total leaf area, our results showed that both growth parameters were higher in lettuce plants from 50-cells plug trays<sup>-1</sup>. However, while RLA increased from amino acids-sprayed plants with both cell plug trays, RLAE only showed significant increases in plants from 288-cells plug trays<sup>-1</sup>.

Higher biomass accumulation on a dry weight basis were found in lettuce plants with the lowest root restrictions (50-cells plug trays<sup>-1</sup>) (Figure 1A). However, different amino acids spray showed significant differences regarding controls in plants from 288-cells plug trays<sup>-1</sup> (Figure 1B), in agreement with Mondal *et al.*, (2015).

Higher RGR and NAR values but lower LAR values from 50-cells plug trays<sup>-1</sup> than those from 288-cells plug trays<sup>-1</sup> were found (Table 3). On the other hand, the positive NAR (Figure 2A), LAR (Figure 2B) and RGR relationships would indicate that the higher biomass accumulation in some amino acids-sprayed lettuce plants would be related with a direct higher photosynthetic capacity (associated with NAR changes) as leaf structure changes which facilitate CO<sub>2</sub> internal mesophyll diffusion (associated with LAR changes) in agreement with Gandolfo *et al.*, (2014). Allometries from roots and shoots let to estimate the photo assimilates partitioning between them. The higher  $\beta$  coefficients in lettuce plants from 288-cells plug trays<sup>-1</sup>



suggested a higher photo assimilates partitioning to roots. On the other hand, plants from 50 cells plug trays<sup>-1</sup> and amino acids-treated plants showed a higher photo assimilates partitioning to shoots (Table 4).

These results set out two questions. The first is related to similar responses found in other vegetables (Pagani *et al.*, 2013; Coro *et al.*, 2014; Di Matteo *et al.*, 2015; Della Gaspera *et al.*, 2016; Geraci *et al.*, 2018) and ornamentals (Di Benedetto and Pagani, 2013; De Lojo and Di Benedetto, 2014; Gandolfo *et al.*, 2014; De Lojo *et al.*, 2017; Molinari *et al.*, 2018; Piotti *et al.*, 2018) where growth has been stimulated in cytokinins-treated plants. A nitrogen availability, cytokinins synthesis and root: shoot ratio relationships have been indicated. However, it is unclear how nitrogen availability is linked with changes in specific cytokinins and how these can change photo assimilates allocation. A feasible hypothesis is

that both root and shoot relative growth rates can be modulated by signals related to both carbon and nitrogen status (Grechy *et al.*, 2007). Cambri *et al.*, (2008) and Biancucci *et al.*, (2015) showed that root elongation is promoted by exogenous supply of proline amino acid, suggested its involvement in the response to abiotic stresses. In the same way, Mano *et al.*, (2012) and Halpern *et al.*, (2015) showed a stimulant growth effect by tryptophan, presumably attributable to auxin synthesis increase. There are only 20 amino acids involved in protein building, but there are 250 more that are known involved in different physiological plant mechanisms (Halpern *et al.*, 2015). Although amino acids are a nitrogen source, the usually concentrations involved in exogenous supply are too low that its positive effects can be attributable to an increase in nitrogen availability.

**Table.1** Total fresh weight at the end of the experiment in lettuce plants from 50- or 288-cell plug tray-1 and sprayed with thirteen amino acids solutions. Different lower case letters indicate significant differences ( $P < 0.05$ ) between control and amino acids-sprayed treatments. Different capital letters indicate significant differences ( $P < 0.05$ ) between plants from different cell plug tray-1

	Fresh weight (g plant <sup>-1</sup> )	
	50-cells	288-cells
<b>Control</b>	62.83bA	50.27bB
<b>Mix</b>	54.48cB	61.00aA
<b>Aspartic acid</b>	60.42bA	35.39 dB
<b>Glutamic acid</b>	55.67cA	49.00bB
<b>Alanine</b>	60.47bA	44.75cB
<b>Cysteine</b>	49.88dA	40.56cB
<b>Glycine</b>	44.22dA	31.46 dB
<b>Leucine</b>	56.47cA	49.83bB
<b>Lysine</b>	67.50aA	49.92bB
<b>Methionine</b>	59.61bA	41.91cB
<b>Threonine</b>	62.15bA	52.71bB
<b>Tryptophan</b>	66.93aA	48.10bB
<b>Valine</b>	57.01cA	43.98cB

**Table.2** Changes in total leaf area, RLAE, RLA and SLA during the experiment for lettuce plants grown in 50- or 288-cell plug tray-1 and sprayed with thirteen amino acids solutions. Different lower case letters indicate significant differences ( $P < 0.05$ ) between control and amino acids-sprayed treatments. Different capital letters indicate significant differences ( $P < 0.05$ ) between plants from different cell plug tray-1. The probability of the slope being zero for RLAE and RLA was  $P < .001$

	Leaf area (cm <sup>2</sup> plant <sup>-1</sup> )		RLAE (cm <sup>2</sup> cm <sup>-2</sup> day <sup>-1</sup> )		RLA (leaves week <sup>-1</sup> plant <sup>-1</sup> )		SLA (cm <sup>2</sup> g <sup>-1</sup> )	
	50-cells	288-cells	50-cells	288-cells	50-cells	288-cells	50-cells	288-cells
<b>Control</b>	1654.05 bA	1532.88 bB	0.0850 cA	0.0766 cB	0.2458 cA	0.2169 hB	35.11 cA	36.50 bA
<b>Mix</b>	1546.27cB	1739.70 aA	0.0840 cB	0.0879 aA	0.2511 bB	0.3053 aA	40.78 bA	32.40 cB
<b>Aspartic acid</b>	1180.00 fA	1184.56 eA	0.0918 aA	0.0747 cB	0.2213 fA	0.1973 iB	25.70 dB	35.86 bA
<b>Glutamic acid</b>	1429.57 dA	1358.73 cA	0.0936 aA	0.0771 cB	0.2222 fB	0.2733 bA	35.26 cA	36.88 bA
<b>Alanine</b>	1816.43 aA	1198.20 eB	0.0904 abA	0.0800 bB	0.2813 aA	0.1484 kB	43.26 aA	37.11 bB
<b>Cysteine</b>	1564.78 cA	1238.90 dB	0.0896 bA	0.0739 cB	0.2347 eB	0.2476 eA	32.56 cB	37.08 bA
<b>Glycine</b>	1376.25 eA	1002.66 fB	0.0878 bA	0.0699 dB	0.2400 dA	0.1707 jB	35.68 cA	33.81 bA
<b>Leucine</b>	1439.36 dA	1318.80 cB	0.0890 bA	0.0789 bB	0.2578 bA	0.2329 gB	35.83 cA	35.78 bA
<b>Lysine</b>	1868.13 aA	1284.00 dB	0.0904 abA	0.0774 cB	0.2524 bA	0.2422 fB	38.10 bA	33.58 bB
<b>Methionine</b>	1656.82 bA	1365.91 cB	0.0917 aA	0.0769 cb	0.2529 bA	0.2573 dA	33.25 cA	33.52 bA
<b>Threonine</b>	1675.36 bA	1420.34 bB	0.0901 abA	0.0788 bB	0.2253 fB	0.2609 cA	35.90 cB	43.62 aA
<b>Tryptophan</b>	1596.15 cA	1323.26 cB	0.0910 aA	0.0759 cB	0.2529 bA	0.2418 fB	34.02 cA	34.41 bA
<b>Valine</b>	1536.38 cA	1461.25 bB	0.0933 aA	0.0727 dB	0.2356 eB	0.2484 eA	32.49 cA	33.27 bA

**Table.3** Changes in RGR, NAR and LAR during the experiment for lettuce plants grown in 50- or 288-cell plug tray-1 and sprayed with thirteen amino acids solutions. Different lower case letters indicate significant differences ( $P < 0.05$ ) between control and amino acids-sprayed treatments. Different capital letters indicate significant differences ( $P < 0.05$ ) between plants from different cell plug tray-1. The probability of the slope being zero for RGR was  $P < .001$

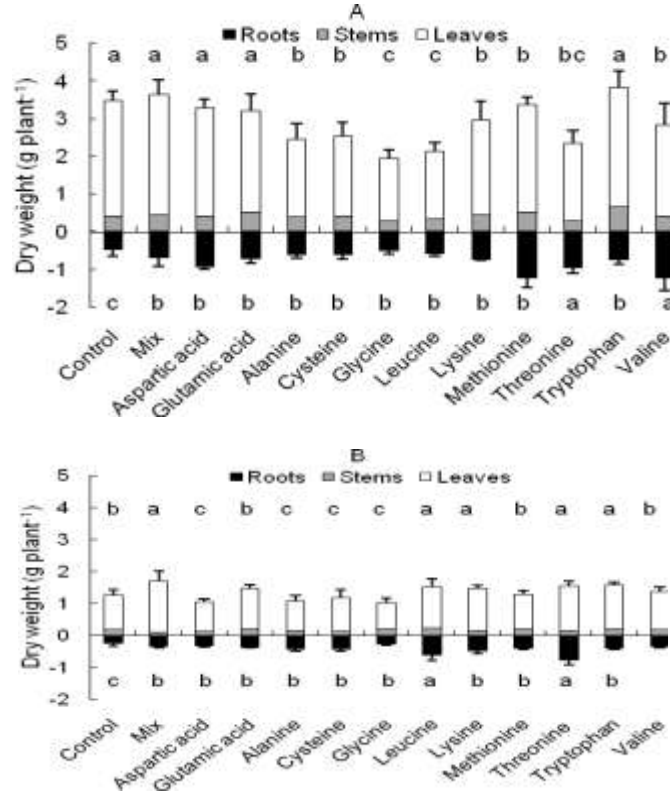
	RGR (g g <sup>-1</sup> day <sup>-1</sup> )		NAR (g cm <sup>-2</sup> day <sup>-1</sup> ) (x 10 <sup>-5</sup> )		LAR (cm <sup>2</sup> g <sup>-1</sup> )	
	50-cells	288-cells	50-cells	288-cells	50-cells	288-cells
<b>Control</b>	0.0799 aA	0.0763 bA	32.60 cA	31.11 aA	245.08 aA	245.23 aA
<b>Mix</b>	0.0772 aB	0.0871 aA	34.38 cA	30.68 aB	224.55 bB	283.92 aA
<b>Aspartic acid</b>	0.0782 aA	0.0739 bA	40.18 aA	29.22 aB	194.61 bB	252.93 aA
<b>Glutamic acid</b>	0.0820 aA	0.0735 bB	39.27 aA	30.34 aB	208.80 bB	242.23 aA
<b>Alanine</b>	0.0776 aA	0.0729 bA	35.30 bA	29.73 aB	219.82 bB	245.18 aA
<b>Cysteine</b>	0.0760 bA	0.0741 bA	39.94 aA	29.02 aB	190.28 bB	255.34 aA
<b>Glycine</b>	0.0778 aA	0.0650 cB	36.91 bA	27.66 aB	210.76 bA	234.98 aA
<b>Leucine</b>	0.0785 aA	0.0749 bA	36.77 bA	30.56 aB	213.51 bB	245.08 aA
<b>Lysine</b>	0.0820 aA	0.0716 bB	33.01 cA	28.13 aB	248.41 aA	254.53 aA
<b>Methionine</b>	0.0764 bA	0.0769 bA	37.45 bA	29.19 aB	204.02 bB	263.41 aA
<b>Threonine</b>	0.0818 aA	0.0747 bB	39.37 aA	29.84 ab	207.78 bB	250.33 aA
<b>Tryptophan</b>	0.0775 aA	0.0750 bA	38.29 aA	27.76 aB	202.41 bB	270.16 aA
<b>Valine</b>	0.0799 aA	0.0747 bA	35.11 bA	27.63 aB	227.58 bB	270.35 aA

**Table.4** Changes in allometric relationships between roots and shoots for lettuce plants grown in 50- or 288-cell plug tray-1 and sprayed with thirteen amino acids solutions. Different lower case letters indicate significant differences ( $P < 0.05$ ) between control and amino acids-sprayed treatments. Different capital letters indicate significant differences ( $P < 0.05$ ) between plants from different cell plug tray-1. The probability of the slope being zero for  $\beta$  coefficient was  $P < .001$

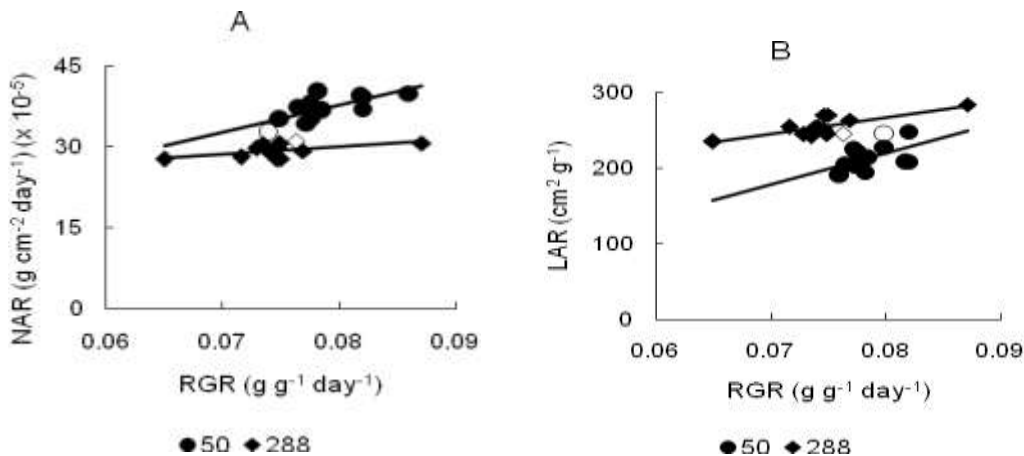
	Root : Shoot ratio		$\beta$	
	50-cells	288-cells	50-cells	288-cells
<b>Control</b>	0.195 dA	0.179 dB	0.782 bA	0.781 aA
<b>Mix</b>	0.191 dA	0.202 dA	0.713 cA	0.704 dA
<b>Aspartic acid</b>	0.280 cA	0.303 cA	0.777 bA	0.800 aA
<b>Glutamic acid</b>	0.219 dA	0.245 dA	0.666 dB	0.752 bA
<b>Alanine</b>	0.252 cB	0.380 bA	0.742 cB	0.825 aA
<b>Cysteine</b>	0.241 cB	0.359 bA	0.712 cA	0.660 eB
<b>Glycine</b>	0.256 cA	0.252 dA	0.723 cB	0.797 aA
<b>Leucine</b>	0.269 cB	0.388 bA	0.641 dB	0.792 aA
<b>Lysine</b>	0.249 cB	0.319 cA	0.711 cB	0.821 aA
<b>Methionine</b>	0.364 bA	0.297 dB	0.750 bB	0.792 aA
<b>Threonine</b>	0.407 aB	0.491 aA	0.773 bB	0.838 aA
<b>Tryptophan</b>	0.192 dA	0.242 dA	0.730 cA	0.703 dA
<b>Valine</b>	0.438 aA	0.252 dB	0.902 aA	0.763 bB



**Fig.1** Dry weight at the end of the experiment on lettuce plants grown in 50- (A) or 288-cell plug tray-1 (B) and sprayed with thirteen amino acids solutions. Different lower case letters indicate significant differences ( $P < 0.05$ ) between control and amino acids-sprayed treatments. Vertical lines indicate standard errors



**Fig.2** NAR (A) and LAR (B) related to RGR for lettuce plants grown in 50- or 288-cell plug tray-1 and sprayed with thirteen amino acids solutions (fully symbols). Control plants: empty symbols. The straight-line regressions were  $NAR_{50} = 497.00 RGR - 2.02$  ( $r^2 = 0.653$ ;  $P < 0.05$ );  $NAR_{288} = 137.65 RGR + 19.02$  ( $r^2 = 0.291$ ;  $P < 0.05$ );  $LAR_{50} = 4182.30 RGR - 114.50$  ( $r^2 = 0.648$ ;  $P < 0.05$ );  $LAR_{288} = 2195.30 RGR + 91.00$  ( $r^2 = 0.579$ ;  $P < 0.05$ )



As nitrate is assimilated into conversion to nitrite, ammonia and then into amino acids, enzymes responsible to cytokinin synthesis are specifically induced by nitrate supply (Miyawaki *et al.*, 2004) which was indicated by the fact the higher nitrogen supply the higher cytokinins concentrations (Takei *et al.*, 2002). A similar pathway control of endogenous amino acids has been suggested by Miller *et al.*, (2002). In the same way, Garnica *et al.*, (2010) showed that nitrates have been related to clear increases in the main active forms of cytokinins and reduction of the levels of the lower active forms, independently of the dose applied. Likewise, the presence of nitrate also enhanced indole acetic shoots content, which correlated with higher cytokinin levels (Pavlikova *et al.*, 2012).

A second point, but not of less importance, is the specific effect of each individual amino acid on plant growth, considering that the most commercial formulations usually included a lot of them. A key view to understand plant carbon-nitrogen relationships is the fact that nitrogen assimilation capacity is lined up to both nutrients availability and requirements through an integrated endogenous signals related to hormones, nitrate, sugars, organic acids and amino acids. Responses to nutrient availability changes have a high plasticity degree and require a precise genic coordination (Liu *et al.*, 2009). From a theoretical point of view, it is extremely difficult to predict what specific amino acid can affect some of the many plant physiological mechanisms (Foyer *et al.*, 2003). In this way our results showed that different single amino acids can change different plant traits such as: fresh weight accumulation (Table 1), dry weight accumulation (Figure 1, Table 3), leaf area expansion (Table 1) and photo assimilates partitioning (Table 4). Lea and Mifflin (2010)

has indicated that the response to an exogenous bio stimulant spray, such as amino acids, is dependent of specie, plant genotype, environment, both formulae concentration and application time. Our present results would be to support it.

In this context, glutamate has been recognized as a signal molecule to plant metabolism (Filleur *et al.*, 2005) and plays a central role in nitrogen metabolism (Schachtman and Shin, 2007). Glutamate has been involved in other minor amino acids metabolism as well. The  $\alpha$ -amino group of glutamate can be transferred to other amino acids (arginine, proline) by the action of multi specific amino transferases (Forde and Lea, 2007). In the same way, Stitt *et al.*, (2002) have indicated that the amino acid biosynthesis pathways are not limited by the availability of glutamate or other amino donors in central metabolism, at least in plants where the nitrogen supplies are not limiting. However, our results showed that one of the amino acids involved in growth positive responses as control plant as mixed-sprayed plants are glutamic acid.

In summary, a single amino acids spray early on nursery would be a tool for improving lettuce yield in the presence of an abiotic stress related to plug cell volume. The use of 288-cells plug tray would need for more than one single amino acid. Although, additive and antagonistic effects between amino acids would be involved, a conclusive results needs for future experiments which would be included wide amino acids combinations and different plant species.

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