Plant response to biowaste soluble hydrolysates in hibiscus grown under limiting nutrient availability

Title

Plant response to biowaste soluble hydrolysates in hibiscus grown under limiting nutrient availability

Daniele Massa[1]* ·Anna Lenzi[2] · Enzo Montoneri[3] · Marco Ginepro[4] · Domenico Prisa[1] · Gianluca Burchi[1]

[1]Research Centre for Vegetables and Ornamentals, Council for Agricultural Research and Economics (CREA), Via dei Fiori 8, 51012 Pescia (PT), Italy

[2]Dipartimento di Scienze delle Produzioni Agrialimentari e dell'Ambiente, Università degli Studi di Firenze, Piazzale delle Cascine 18, 50144 Firenze, Italy

[3]Biowaste Processing, Via XXIV Maggio 25, 37126 Verona, Italy

[4] Dipartimento di Chimica, Università di Torino, Via P. Giuria 7, 10125 Torino, Italy

*Corrisponding author:

Daniele Massa

Tel +39 0572 451033

Fax +39 0572 453309

daniele.massa@crea.gov.it

Abstract

Biostimulants are substances promoting plant growth, quality and stress resistance. The present work aimed to investigate whether soluble hydrolysates from biowaste performed as biostimulants. Hibiscus (*Hibiscus moscheutos* L. subsp. *palustris*) plants were subjected to four treatments: standard fertilization, low fertilization, and low fertilization with added soluble digestate or soluble compost. Plant performance indicators were biomass accumulation, biometric parameters, leaf gaseous exchanges and elemental composition, and nitrogen-use efficiency. Low fertilization negatively affected most of the investigated parameters. However, plants treated with biowaste-derived products performed better than untreated low-fertilized plants (+21 to 145 % for biomass accumulation and biometric parameters, and carbon assimilation rate) and for many parameters reached values comparable to those showed by standard-fertilized plants or even higher, as in the case of K, Si, and Mo leaf content, and N use efficiency. Therefore, the tested soluble hydrolysates demonstrated to have biostimulant properties in hibiscus grown under nutritional stress.

Keywords

Biostimulants, *Hibiscus moscheutos*, Nutrient stress, Photosynthesis, Substrate cultivation

1 Introduction

In the last decade, much attention has been focused on the use of biowaste-sourced products as biofertilizers for eco-friendly sustainable agriculture (Pirdashti et al., 2010; Sortino et al., 2014). However, a new class of products named biostimulants is emerging (Calvo et al., 2014). Biostimulants are “substances promoting plant growth without being nutrients, soil improvers, or pesticides” (du Jardin, 2015). They stimulate the physiology of plants, promoting their growth and enhancing their stress resistance. Generally, biostimulant substances enhance plant growth and quality (Calvo et al., 2014). In more detail, they have improved plant photosynthesis (Castro et al., 2012), nutrition (Saa et al., 2015), and other physiological processes (Bulgari et al., 2015). Compared with biofertilizers, the capacity of biostimulants to promote plant growth under stressful conditions is the main distinguishing factor (Petrozza et al., 2014).

Soluble bio-based substances isolated from the alkaline hydrolysate of fermented urban biowastes have performed as efficient promoters of growth and productivity of food (Sortino et al., 2014) and ornamental plants (Fascella et al., 2015). These substances contain 72-85 % organic matter and 28-15 % mineral matter, depending on the type of sourcing biowaste, and are rich in plant nutrients. The organic matter is composed of a mix of molecules with molecular weight from 5 to several hundreds kDa (Montoneri et al., 2011). It comprises aliphatic and aromatic C atoms bonded to a variety of acid and basic functional groups, which bind and keep in solution the mineral elements. Sortino et al. (2014) have suggested that, due their capacity to complex Fe ions and keep them in solution at slightly acidic or alkaline conditions, the above soluble, biobased substances may contribute to enhance photosynthesis, and in turn plant growth and yield. On these bases, the support of ecosystems based on cycling renewable organic C between wastes and added value products appears as fascinating reachable goal, certainly worthwhile to pursue.

Intensive cultivation of potted ornamentals represents one of the most specialized growing systems in agriculture. It stands out for the high use of agrochemicals per unit area necessary to avoid any possible stress, thereby ensuring high aesthetic quality level (Kader, 2000). The possibility of boosting ornamental crops in intensive production systems with organic biowaste gives at the same time interesting perspectives related to organic matter recycling and improved input use efficiency in agriculture. In other works on ornamental crops (Fascella et al., 2015; Massa et al., 2016), the above biowaste soluble hydrolysates were suggested to have potential biostimulant performance. This hypothesis was based upon the fact that their effects on several indicators of plant growth and flower production were similar to or better than the effects exhibited by commercial biostimulants, which were tested for comparison. In the above-mentioned works, plants were cultivated in optimal growing conditions, but biostimulant substances are known for improving plant performance under biotic and abiotic pressures (Calvo et al., 2014; Bulgari et al., 2015; du Jardin, 2015). Therefore, a step forward consists in further experimentations carried out in presence of limiting growing conditions, as proposed by other authors (Anjum et al., 2011; Ertani et al., 2013). The present work reports the effects of the above mentioned soluble hydrolysates on hibiscus, selected as test plant, grown under nutritional stress conditions. To the authors' knowledge, no previous studies have been published on the effects of urban biowastes hydrolysates on the cultivation of ornamental plants grown in substrate under nutrient stress. Yet, in view of the worldwide easy availability of urban biowastes, investigating the full potential of these materials as source of products for use in agriculture is highly worthwhile.

2 Materials and Methods

2.1 Experimental site and growing conditions

The experiment was carried out from April to July 2014, under typical Mediterranean climate conditions, at the Landscaping Plants and Nursery Research Unit of the Italian

Council for Agricultural Research and Economics, Pescia, Tuscany, Italy (lat. 43°54' N, long. 10°42' E). During the experiment, radiation, relative humidity, and air temperature were recorded every five minutes through an on-site meteorological station (Dacagon Device, Pullman, WA 99163 USA). Minimum, mean and maximum daily averaged photosynthetic photon flux density was 109.2, 568.3, and 750.5 µmol m^{-2} s^{-1}, respectively. Mean daily cumulated global radiation was 21.7 MJ m^{-2} d^{-1}. Average of minimum, mean and maximum daily air temperature was 11.6, 20.5 and 22.3 °C, respectively. Air mean daily relative humidity averaged 64.5 %.

Hibiscus seedlings (*Hibiscus moscheutos* L. subsp. *palustris*) were transplanted into 4-L black polyethylene pots (18 cm diameter) on 10 April 2014 using a mixture of peat and pumice (1:1 V:V) adjusted to pH 6 by calcium carbonate. Average shoot dry biomass and leaf area of the seedlings were determined. Pots initially were placed under 40 % shading net for plant acclimatization to outdoor conditions. Thirty days after transplanting, they were moved to the open-air and arranged in a randomized, complete block experimental design, with three replicates per treatment (eight plants per replicate). A 0.40 x 0.60 m spacing was adopted, resulting in a crop density of 4.2 plants m^{-2}. On 30 May 2014 all plants were trimmed above the fourth true leaf to stimulate the emission of lateral shoots, as recommended by the standard production technique (PianteMATI™, personal communication, February 2014). Plants were irrigated by drip irrigation. Irrigation was triggered by a timer whose schedule was adjusted weekly according to climate condition and leaching fraction. Leaching fraction was calculated as the ratio between drainage water, measured in three pots per block, and supplied water. Irrigation was then regulated in order to keep a constant leaching fraction value. Irrigation water pH and electrical conductivity ranged from 6.2 and 6.6, and from 0.42 and 0.60 dS m^{-1}, respectively. The experiment ended 20 weeks after transplant, one month before the presumable beginning of plant senescence based on local climate conditions.

2.2 Biowaste material

The investigated soluble hydrolysate products were produced and supplied by Studio Chiono ed Associati in Rivarolo Canavese, Torino, Italy. The pristine materials were the digestate recovered from the anaerobic fermentation of the organic humid fraction of municipal solid waste from separate source collection, and the green compost was obtained by over 180 days aerobic fermentation of private gardening and public park trimmings. The digestate and the compost were processed as described by Sortino et al. (2014) to obtain the final dried products, which hereinafter will be named soluble digestate and soluble compost, respectively, or soluble hydrolysates if mentioned together.

The chemical characteristics of the final products are shown in Table 1. Moreover, the organic matter was characterized by the following C types and functional groups content values (C mmol g^{-1} dry matter) for the soluble digestate and the soluble compost, respectively: aliphatic C 14.2 and 12.0, amine C 3.3 and 2.2, methoxy C 1.3 and 0.0, alkoxy C 3.3 and 4.5, anomeric C 0.97 and 1.3, aromatic C 3.3 and 4.2, phenol C 0.66 and 1.6, phenoxy C 0.33 and 0.65, carboxylic acid C 2.3 and 3.9, amide C 3.0 and 0.33, ketone C 0.33 and 1.5.

2.3 Treatments

Plants were subjected to four different fertilization treatments (Table 2): 1) standard fertilization (SF); 2) low fertilization (LF); 3) low fertilization with soluble digestate (LFSD); 4) low fertilization with soluble green compost (LFSGC).

Fertilization was administered in part by two controlled-release fertilizers (Osmocote Pro® 3-4 and Osmocote Pro® 5-6 months in the same amount) mixed with the substrate (Table 2). In addition, soluble fertilizers (for SF and LF) or soluble hydrolysate products (for LFSD and LFSGC) were dissolved in the irrigation water and supplied in the cultivation

period between exponential growth and incipient flowering (from 70 to 80 days after transplant) (Table 2).

In LFSD and LFSGC treatments, the amounts of soluble digestate and soluble green compost, respectively, were calculated considering the chemical composition of the two products (Table 1) in order to provide plants with the same amount of organic matter (i.e. 0.65 kg m^{-3}).

Standard fertilization (SF) was intended to avoid any nutritional stress while low fertilization (LF) was intended to produce nutrient stress in plants. The LF, LFSD and LFSGC treatments were arranged to receive comparable amounts of N, P, and K (Table 2).

With regard to the other macronutrients, S was supplied with sulphuric acid used for adjusting water pH at 5.5-6.5 during irrigation, while Ca and Mg were naturally present in the irrigation water at considerable concentrations (i.e. roughly 1.00 and 0.25 mol m^{-3}, respectively). On the contrary, the presence of micronutrients in the irrigation water was deemed negligible. Therefore, micronutrients were also strongly reduced in low-fertilized plants.

2.4 Plant analyses

Leaf gas exchange measurements were performed one week before the destructive analysis, between 9.00 and 12.00 am (Fini et al., 2010), by a portable photosynthesis system (Ciras-2, PPSystems, Amesbury, MA 01913 USA). During measurements, to maintain comparable analytical conditions, the chamber was set at a constant value of light suturing photosynthesis (1000 µmol m^{-2} s^{-1}, primarily determined through photosynthesis light-response curves), CO_2 (400 ppm), vapour pressure deficit (1.0 ±0.2 kPa), and temperature (27.5 ±0.9 °C). The operating temperature was calculated as the average of the temperature values recorded with a datalogger, in the same daily period of measurements, during the three days before the beginning of measurements. Two mature and healthy leaves

(second and fourth completely unfolded leaf above the apex of the main stem) per plant were chosen for gas exchange analysis in six plants per treatment (two plants per replicate; 12 measurements per treatment). The analysis provided current net photosynthetic (carbon assimilation) rate, transpiration rate and stomatal conductance values.

At the end of the experiment, the following parameters were determined, by destructive analyses, on four plants per replicate (12 plants per treatment): shoot fresh and dry biomass production, plant height, plant volume, leaf area, leaf SPAD index, and mineral composition of plant tissues. Fresh and dry biomass weight were measured before and after oven-drying at 80°C for 72 h. Plant volume was calculated as the volume of an ellipsoid after measuring plant height and mean diameter of the canopy projected to the soil. Leaf area was measured through a leaf area meter (WinDIAS Image Analysis System, Delta-T Devices, U.K.). Leaf chlorophyll content was measured through a portable SPAD-502 (Konica Minolta Optics, 2970 Ishikawa-machi, Hachioji, Tokyo, Japan) as reported by Massa et al. (2016). Shoot dry biomass and leaf area were used to calculate plant growth indices, i.e. relative growth rate (mg g^{-1} d^{-1}), net assimilation rate (g m^{-2} d^{-1}), and leaf area ratio (m^2 kg^{-1}).

Nitrogen was determined in the shoot (separately for leaves, stems and flowers) by a Kjeldhal method (Massa et al., 2016). Leaf tissues were analysed for all other macro and micronutrients, beneficial elements (i.e. Al, Si, Na, Co), and heavy metals (i.e. Cr and Pb), through inductively coupled plasma analysis, after microwave digestion. Nitrogen use efficiency indices were calculated as follows: i) physiological use efficiency as the ratio between shoot dry biomass and total amount of N in shoot tissues; ii) agronomic use efficiency as the ratio between shoot dry biomass and total N supplied with fertilizers; iii) recovery efficiency as the ratio between the total amount of N in shoot tissues and the one supplied with fertilizers.

2.5 Statistics

Collected data were analysed through one-way ANOVA to assess significant differences among treatments at 95 % of probability. Mean values were separated by Duncan's multiple range test (P = 0.05) (Duncan, 1955). Correlation analysis among measured parameters was also performed.

3 Results

3.1 Plant biomass accumulation and biometric parameters

Table 3 presents a comparison of the standard-fertilized plants and starved plants for biomass accumulation and biometric parameters. The data show that, compared with standard fertilization, the plants grown in the low-fertilized substrate with no added soluble hydrolysates (LF) gave significantly lower values for all indicators. The latter plants exhibited the following relative decreases: 49 and 47 % for fresh and dry biomass, respectively, 43 % for leaf area, 19 % for plant height, and 58 % for plant volume. Plants treated with soluble hydrolysates showed significant improvements of most of plant performance indicators compared with untreated plants (LF). Fresh biomass, accumulated at the final destructive analysis, was increased by adding soluble digestate or soluble compost by 35 and 65 % and the dry biomass increases accounted for 37 and 83 %, respectively, compared with the LF treatment without soluble substances.

Plant relative growth rate reflected the dry biomass accumulation pattern observed in the different treatments (Table 3). On the contrary, the net assimilation rate and leaf area ratio of plants treated with the soluble digestate was comparable with low-fertilized plants without the addition of soluble hydrolysates.

The soluble digestate and soluble compost significantly enhanced not only the already mentioned leaf area (+57 % on average), but also plant height (+22 % on average) and volume (+135 % on average), compared with the low fertilization treatment in the absence of

soluble hydrolysates. Moreover, no significant difference was observed in plant height or volume between plants grown in the low-fertilized substrate with added soluble hydrolysates and standard-fertilized plants (Table 3).

3.2 Leaf gaseous exchange activity and chlorophyll

The limited nutrient availability in the root zone affected negatively the maximum photoassimilation capability of starved plants (Table 2), which thus showed a significant reduction in the net photosynthetic rate measured at saturating light (Figure 1). In spite of the 23 % reduction in photosynthetic rate observed for LF treatment plants compared with the standard-fertilized control, the addition of soluble hydrolysates caused 28 % increase of leaf carbon intake reaching the same level shown by standard-fertilized plants. These results were consistent with data collected on leaf chlorophyll content (SPAD index; Figure 1). Similar trends were also observed for the stomatal conductance, while the transpiration rate in standard-fertilized plants was significantly higher than in LF plants but lower than in plants treated with soluble digestate or soluble compost (Figure 1). As a consequence of the above results, the latter treatments showed the lowest leaf water use efficiency values (data not shown). The higher transpiration rate observed in these treatments was consistent with the increased dry matter percentage in plant tissues (data not shown).

3.3 Leaf nutrient concentration and nitrogen use efficiency

Table 4 reports the leaf mineral content in plants grown under the different fertilization treatments. The concentrations of three over six analysed macronutrients were enhanced by the application of the soluble hydrolysates compared with the low fertilization treatment in their absence. The latter showed the lowest content in P and K among all treatments. For some elements plants treated with soluble hydrolysates showed the same concentration than standard-fertilized plants, for example for N and Al, or even a higher content as in the case of K, Ni and Si (Table 4).

In more detail, N concentration was significantly reduced by 13 %, on average, in the low-fertilized plants with no added soluble hydrolysates (LF) and with the addition of soluble digestate (LFSD), compared with the standard-fertilized plants. Conversely, the soluble compost treatment (LFSGC) enhanced N concentration in plant leaves, thereby resulting not statistically different from the standard fertilization.

Phosphorus was significantly reduced (-23 % on average) by the nutrient starvation due to low fertilization compared with the standard-fertilized treatment. However, plants treated with both the soluble digestate and the soluble compost exhibited significantly higher P compared with the low-fertilized plants with no added soluble hydrolysates.

Starved plants not treated with soluble hydrolysates exhibited the lowest value also for K, whose concentration was 22 % lower than in the standard-fertilized plants. On the contrary, both the soluble digestate and the soluble compost increased significantly K concentration above the value observed in the standard-fertilized plants.

Among the other macronutrients, Ca, Mg, and S gave different responses. The former two elements were slightly, although significantly, reduced in the leaves of plants treated with the soluble hydrolysates. Moreover, sulphur was reduced in the starved plants, both in presence and absence of soluble hydrolysates, compared with the standard-fertilized control plants.

The different treatments did not significantly influence leaf concentration in Fe, Mn, B, and Zn (Table 4) and other micronutrients (data not shown) with the exception of Mo and Ni. Molybdenum was higher by 58 %, on average, in all starved plants compared with the standard-fertilized plants. The addition of the soluble compost (LFSGC) caused an increase in Ni by 53 % compared with the average concentration observed in the plants subjected to the other treatments.

All measured beneficial elements (excluding Co) were increased by the soluble compost treatment compared with the other two low fertilization treatments. Particularly remarkable was the 39-48 % increase of Si concentration in plants treated with the soluble compost (LFSGC) compared with the plants subjected to all the other treatments. Silicon trend in leaf tissue reflected its concentration in the soluble hydrolysates; in fact, in the soluble compost this element was 72 % higher than in the soluble digestate (Table 1).

Figure 2 shows data regarding the three N use efficiency indices. It can be observed that the plants grown in the low-fertilized substrate in the absence of soluble hydrolysates and in the presence of soluble digestate exhibited significant 17 % higher N physiological use efficiency compared with the other plants. Data collected on N agronomic use efficiency shows that the soluble digestate, and more so the soluble compost, significantly enhanced the agronomic use efficiency of N, by 62 and 117 %, respectively, compared with the average of the two other treatments. Similar effects and differences among treatments were observed for the N recovery use efficiency (i.e. 50 and 134 % increase, respectively).

4 Discussion

Plants grown in the low-fertilized substrate with no added soluble hydrolysates showed significantly lower values for all the growth parameters. These findings were fundamental to validate the basic assumption of the experimental plan adopted in the present work, i.e., that the lower nutrient amount provided to the low-fertilized substrates (Table 2) could induce plant nutritional stress. In this case, plant response was consistent with expectations based on literature reporting the effect of nutrient limitation on cultivated plants (Maathuis, 2009; Marschner, 2011). Crops respond to low nutrient availability in the root zone by decreasing growth and yield with hyperbolic patterns. Below a certain threshold, the lower is the nutrient concentration, the lower is the nutrient uptake rate (Massa et al., 2009) and the higher is the consumption of energy to improve nutrient uptake capability through active ion transport

mechanisms (Marschner, 2011). Limited availability of nutrients induces feed-back mechanisms on plant growth and development, and on secondary and primary metabolic processes, such as photosynthesis that in turn reduces carbon assimilation (Nagarajan and Smith, 2012).

However, in the plants supplied with soluble hydrolysate the dry biomass accumulation was higher than with untreated plants (LF treatment) denoting an improved capability of the formers in facing low nutrient availability in the root zone, especially for those plants treated with the soluble compost (LFSGC). These data were consistent with the estimated relative growth rate. On the contrary, the net assimilation rate of plants treated with the soluble digestate (LFSD) was comparable with low-fertilized plants without the addition of soluble hydrolysates. Therefore, the higher relative growth rate and dry biomass accumulation observed for these plants, compared with the low fertilization treatment in the absence of soluble hydrolysates, was related mainly to the slightly higher leaf area (higher surface for photoassimilation), leaf area ratio, and the significantly higher net photosynthetic rate. Positive effects of the soluble hydrolysates were observed also on the biometric parameters. Since plants grown in the low-fertilized substrate with no added soluble hydrolysates and those treated with soluble digestate or compost underwent the same agronomic conditions (i.e. substrate, fertilization, irrigation, and climate), the better performance of the two latter treatments could be explained by the presence of substances able to stimulate or improve plant response to nutrient stress.

Many authors report benefits obtained on crops treated with products derived from organic substances, including plant growth, yield and quality (Verlinden et al., 2009; Calvo et al., 2014; Bulgari et al., 2015; du Jardin, 2015). The biowaste derived products used in this work belong to the category of “complex organic material”, as proposed by du Jardin (2015) and have structural similarities with natural humic substances (see methodology).

Considering the biomass production and the limitation in nutrient supply reported in this study, the biomass accumulation of plants grown in the presence of the soluble hydrolysates was appreciable. However, the high dry weight observed for the soluble compost treatment, in comparison with the other treatments, was mainly due to the higher biomass accumulated into the stems.

The above discussed findings were consistent with leaf gas exchange measurements. From early studies, K limitation in the root zone decreased net photosynthesis due to its key role in RuBisCO biosynthesis and chloroplast light use efficiency, as well as in stomatal activity (Barker et al., 2007; Marschner, 2011). At different extents, the shortage of N and P limit the formation of many metabolites involved in photosynthesis (e.g., protein, ATP, etc.) thereby decreasing directly and/or indirectly (feed-back mechanisms) carbon assimilation processes (Barker et al., 2007; Marschner, 2011).

The enhanced photosynthetic capacity observed in plants treated with soluble hydrolysates was likely the main variable accounting for the higher biomass accumulation in comparison with low-fertilized plants with no added product. The former plants showed also higher stomatal conductance that in turn is related to high gaseous exchange activity in the mesophyll, which is fundamental for fast carbon turnover into the chloroplasts (Medrano et al., 2002). However, photoassimilate conversion process into organic matter appeared more efficient for soluble compost (LFSGC) than for soluble digestate (LFSD), which showed lower dry biomass accumulation and net assimilation rate. Indeed, different carbon assimilation can be ascribed to different primary metabolic functions not investigated in this work but able to influence the actual carbon storage in structural tissues (Herms and Mattson, 1992). Data collected on photosynthetic activity were consistent with the results observed for leaf chlorophyll (SPAD index) content. On the other hand, SPAD index has been found having a valuable role for assessing plant quality of ornamental species since it is correlated

to leaf greenness and the health general status of the plant (Loh et al., 2002). The enhancement of photosynthesis and chlorophyll content ascribable to biostimulant organic substances has already been reported by other authors for ornamental (Fan at al. 2014; Fascella et al., 2015) and other horticultural species (Sortino et al., 2014; Colla et al., 2015). Indeed, the use of products that stimulate photosynthesis is of great interest in intensive cultivation systems.

The soluble hydrolysates showed high capability in improving plant nutrition and mineral element accumulation under nutritional stress. This aspect was not evaluated in previous works with similar products (Fascella et al., 2015). In more details, the soluble compost treatment enhanced N concentration in plant leaves with results not statistically different from the standard fertilization; this result occurred in spite of the fact that the soluble digestate contained 48 % more N than the soluble compost (see Table 1). Therefore, the latter treatment appeared more efficient in promoting N uptake and organication than soluble digestate. Several works show increased N metabolisms in plants biostimulated with different organic substances (Verlinden et al., 2009; Calvo et al., 2014; Hernandez et al., 2014). Phosphorus starvation has been reported to limit P concentration in leaf tissue especially when coupled with N and K deficiency (Marschner, 2011). On the other hand, the presence of soluble hydrolysates enhanced P content in leaves. A number of works report higher P availability for plants treated with humic-like substances (e.g. Verlinden et al., 2009) since they may prevent calcium phosphate precipitation in the root zone (Calvo et al., 2014; du Jardin, 2015). Extra P availability in leaves is essential for those plants that show improved net carbon intake due to the up-regulation of photorespiratory pathway and alternative electron flow (Huang et al., 2014). However, the most remarkable nutrient intakes caused by soluble hydrolysates occurred with K and were even higher than in well-fertilized plants. This was consistent with the increased photosynthesis rate and chlorophyll content. In

fact, K is a key element to face abiotic stresses due to its crucial role in carbon assimilation, RuBisCo biosynthesis, stomatal activity, and ATP use efficiency (Cakmak, 2005; Barker et al., 2007). Data observed for Ca and Mg supported the hypothesis that higher K intake is coupled to lower Ca and Mg uptake into the symplast (Li et al., 2013). Sulphur concentration in leaf tissues was correlated positively with P ($P \leq 0.001$; $r = 0.95$; $n = 12$). Very little is reported in literature on S response in biostimulated plants, especially in nutritional stress conditions (Calvo et al., 2014). These findings could provide new insights into plant nutrition of biostimulated plants showing a possible relationship between P and S uptake in hibiscus.

Looking at the micronutrient content, only Mo and Ni were influenced by the different treatments. In presence of N depletion in the root zone, Mo uptake has been found increasing due to its key role in the conversion process of NO_3 to NH_4 (Marschner, 2011). This evidence was in agreement with the higher Mo concentration observed in this works for starved plants. Various hypotheses have been formulated on the possible role of Ni in contrasting environmental stress by increasing antioxidant metabolism (Fabiano et al., 2015).

For all investigated beneficial elements, with the exception of Co, a higher concentration was found in leaf tissue of plant treated with soluble compost (LFSGC). These elements are supposed improving many physiological functions in higher plants depending on solubility, pH, and on the interactions with various ions and organic molecules (Pilon-Smits et al., 2009; du Jardin, 2015). Among mineral beneficial elements, indeed Si represents one of the most studied and effective plant biostimulant (Savvas and Ntatsi, 2015), in which tested product were rich (see Table 1). This element has been found increasing net carbon assimilation by improving plant photosynthesis (Chen et al., 2011), and increasing leaf chlorophyll (Pilon et al., 2013). Generally, these effects result in increased biomass accumulation (Savvas and Ntatsi, 2015). An enhancement in Al and Na uptake (when present at low concentrations in the root zone) is typically associated to higher Si availability (Savvas

and Ntatsi, 2015). Moreover, Si has been found improving P and K uptake in presence of nutrient stress and reducing Ca uptake (Mehrabanjoubani et al., 2015). Most of these effects are evident within the data reported in the present study. The higher performance of the soluble compost compared with the soluble digestate may be also due to relatively higher content in aromatic C, phenol and acid functional groups (see methodology). These are likely able to interact in different ways with the mineral elements, and thus differently influence their availability and transport mechanism from the cultivation substrate to the plant and within the plant in the different organs (du Jardin, 2015). However, it should be highlighted that plants grown with soluble hydrolysates were supplied with a higher total amount of micronutrients, due to hydrolysate chemical characteristics (see section 2.3 and Table 1), than untreated (LF) plants, which could have improved plant nutrition and growth.

Plants grown in the low-fertilized substrate in the absence of soluble hydrolysates and in the presence of soluble digestate exhibited the highest values in N physiological use efficiency. Yet, these plants were characterized by lower biomass fresh and total weight. Such a plant response is the well known consequence of a suboptimal N intake: plants react to nutritional stress spending much energy in nutrient utilization processes and limiting luxury consumption (Richard-Molard et al., 2008). These results would support the hypothesis that, compared with the soluble digestate, the soluble compost improved nutrient uptake efficiency, thus allowing higher nutrient availability at plant level and reducing the consumption of energy possibly addressed to overcome nutrient stress. Therefore, the soluble digestate appeared less efficient than the soluble compost in contrasting the nutritional stress at the whole plant level. As matter of fact, this treatment showed a lower net assimilation rate than the soluble compost.

Finally, the results reported for N agronomic and recovery use efficiency are highly relevant in relation to the economic sustainability and the environmental impact of agriculture

practices, particularly those intended for boosting intensive production of ornamental crops. Definitely, modern agriculture implies an efficient use of fertilizers, in order to increase growers' incomes and reduce crop environmental impact since all nutrients not absorbed by the crop potentially become waste and pollutants (Mekonnen and Hoekstra, 2011).

5 Conclusions

For the first time, the soluble hydrolysates obtained from urban biowastes, studied in this work, and already successfully used in different experimental conditions, were demonstrated to have biostimulant and nutritional properties on ornamental plants grown under nutritional stress. The soluble compost proved to be more efficient than the soluble digestate in boosting plant performance of hibiscus grown in intensive substrate cultivation system. Considering that the substrate treated with the soluble hydrolysates was supplied with reduced amount of chemical N, P and K (and micronutrients) than the standard-fertilized substrate, the performance of the plants grown in the soluble compost-treated substrate was remarkable. The results are useful from the economic and environmental point of view for both agriculture sustainability and management of urban biowastes.

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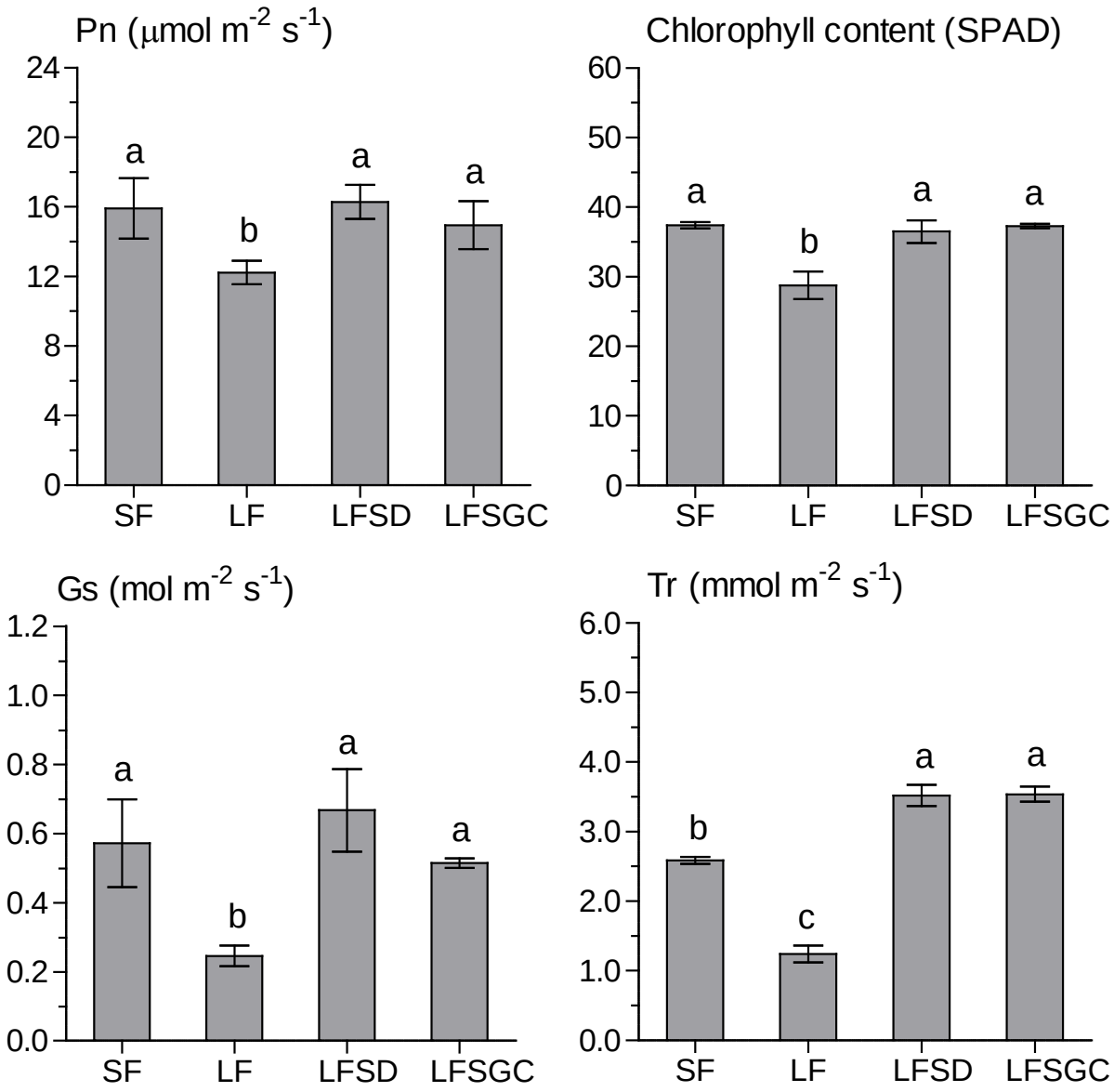
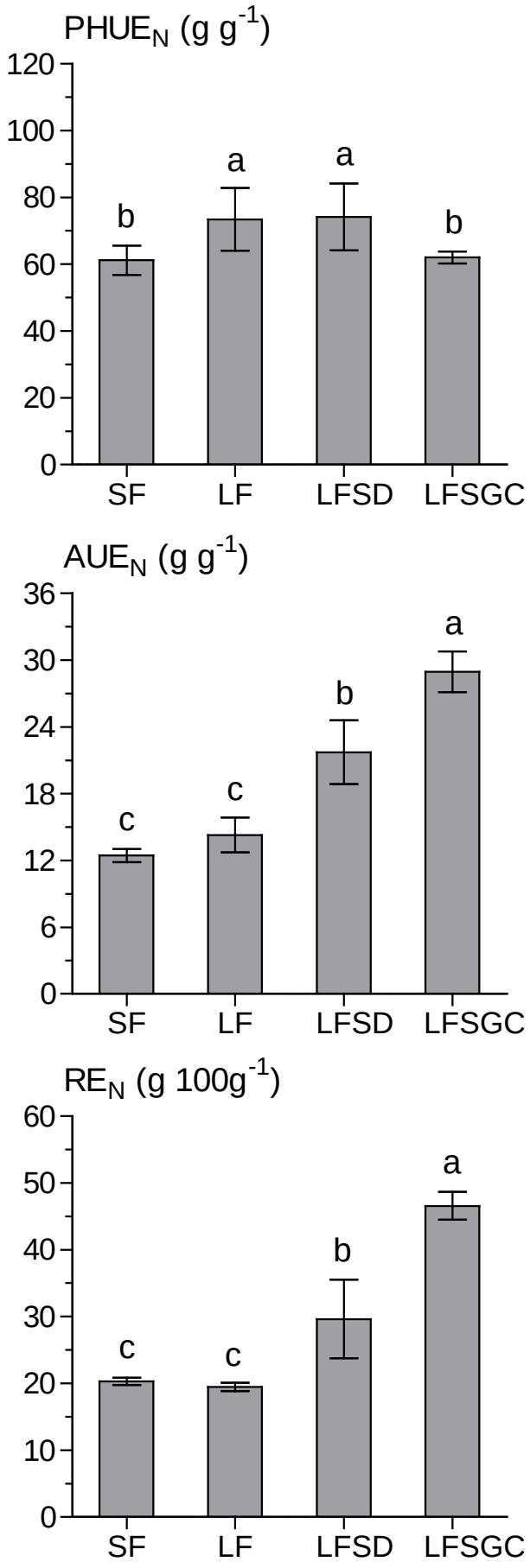


Figure 1 Leaf net photosynthesis rate (Pn), chlorophyll (SPAD index) content, stomatal conductance (Gs) and transpiration (Tr) of *Hibiscus* grown in substrates with different fertilization treatments: standard fertilization (SF), low fertilization (LF), and LF with added soluble digestate (LFSD) or soluble compost (LFSGC). Gaseous exchanges were measured at saturating light and constant temperature, carbon dioxide and humidity in the cuvette of the gaseous exchange analyser. Each ordinate value represents the mean of replicates ± standard deviation (n = 3). Different letters indicate significant differences according to Duncan's multiple-range test (P = 0.05).


PHUE$_N$ (g g^{-1})
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36
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Figure 2 Physiological use efficiency ($PHUE_N$), agronomic use efficiency (AUE_N), and recovery efficiency (RE_N) of nitrogen (N) of *Hibiscus* grown in substrates with different fertilization treatments: standard fertilization (SF), low fertilization (LF), and LF with added soluble digestate (LFSD) or soluble compost (LFSGC). Each ordinate value represents the mean of replicates ± standard deviation (n = 3). Different letters indicate significant differences according to Duncan's multiple-range test (P = 0.05).

Table 3

	Growth parameters				
	Total fresh weight(g m^{-2})	Total dry weight(g m^{-2})	Leaf area(cm^2 pt^{-1})	Plant height(cm pt^{-1})	Plant volume(cm^3 pt^{-1})
SF	911a	255a	2543a	41.5a	44.2a
LF	465d	135c	1459c	33.5b	18.5b
LFSD	627c	185b	2268b	41.5a	41.7a
LFSGC	772b	246a	2303ab	40.5a	45.4a
p-value	0.002	<0.001	<0.001	0.008	0.005
	Growth indexes				
	Relative growth rate(mg g^{-1} d^{-1})		Net assimilation rate(g m^{-2} d^{-1})		Leaf area ratio(m^2 kg^{-1})
SF	28.4a		6.8a		4.2bc
LF	23.5c		5.3b		4.5ab
LFSD	25.9b		5.3b		4.9a
LFSGC	28.1a		7.0a		4.0c
p-value	<0.001		0.004		0.017

Table 4

					Macronutrients (g kg^{-1})	
	N	P	K	Ca	Mg	S
SF	24.6a	3.6a	21.4c	15.3a	5.7a	5.2a
LF	21.4b	2.6c	16.7d	15.5a	5.8a	2.8c
LFSD	21.3b	3.0b	22.5b	15.0b	5.2bc	4.0b
LFSGC	23.0ab	2.7bc	23.0a	13.8c	4.9c	2.7c
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
					Micronutrients (mg kg^{-1})	
	Fe	Mn	B	Zn	Mo	Ni
SF	163	701	40.0	29.9	0.6b	0.9b
LF	134	800	55.7	38.5	1.0a	0.7b
LFSD	96	877	48.4	29.7	1.0a	0.9b
LFSGC	110	774	49.4	33.3	0.9a	1.2a
p-value	n.s.	n.s.	n.s.	n.s.	0.009	0.002
				Beneficial elements and non-nutrient heavy metals (mg kg^{-1})		
	Al	Si	Na	Co	Pb	Cr
SF	46.6a	316.2b	657b	0.4	1.1	0.3
LF	34.7b	310.8b	574b	0.5	0.9	0.3
LFSD	34.6b	297.0b	596b	0.4	1.1	0.3
LFSGC	50.6a	438.2a	866a	0.5	0.9	0.4
p-value	<0.001	<0.001	<0.001	n.s.	n.s.	n.s.

Table 1. Analytical data for the soluble digestate (SD) and compost (SGC) substances used in the experimental trial.

	Electrical conductivity (EC; dS m^{-1}), Dry matter, Ash, organic matter (OM), and non-metal elements (g $100g^{-1}$)										
	EC	Dry matter	Ash	OM	C	N	P	Si	pH	C/N	OM/(C+N)
SD	6.4 ± 0.1	99.4 ± 0.1	15.4 ± 0.2	66.4 ± 0.9	40.0 ± 0.4	6.63 ± 0.08	0.50 ± 0.04	0.25 ± 0.03	10.5 ± 0.1	6	1.4
SGC	8.2 ± 0.1	97.3 ± 0.1	27.9 ± 0.6	64.6 ± 1.3	39.0 ± 0.5	4.49 ± 0.21	0.23 ± 0.02	0.43 ± 0.01	10.1 ± 0.2	8.7	1.5

	Metal elements (g $100g^{-1}$)						Metal elements (mg $100g^{-1}$)				
	K	Ca	Mg	Fe	Al	Na	Cu	Ni	Zn	Cr	Pb
SDSGC	1.59 ± 0.06	2.08 ± 0.05	0.27 ± 0.01	0.52 ± 0.00	0.10 ± 0.04	0.19 ± 0.01	262 ± 1	24 ± 1	361 ± 4	15 ± 0	46 ± 2
	2.12 ± 0.21	2.86 ± 0.38	0.38 ± 0.06	0.83 ± 0.04	0.13 ± 0.04	0.30 ± 0.01	264 ± 4	91 ± 1	303 ± 1	32 ± 1	65 ± 1

Each value reported in the table represents the mean of replicates ± standard deviation (n = 3).

Table 2. Controlled-release fertilizers (CRF), soluble fertilizers (FERT), soluble digestate (SD), and soluble green compost (SGC) used in the different treatments. Total nitrogen (N), phosphorus (as P_2O_5), and potassium (as K_2O) supplied in the four treatments are also reported.

Treatment	Product (kg m^{-3})						Total nutrient supply (kg m^{-3})		
	CRF	FERT			SD	SGC	N	P_2O_5	K_2O
		N	P_2O_5	K_2O					
Standard fertilization (SF)	6.0	0.21	0.28	0.13	0	0	1.2	0.8	0.7
Low fertilization (LF)	3.0	0.05	0.01	0.02	0	0	0.6	0.3	0.3
Low fertilization with soluble digestate (LFSD)	3.0		0		0.99	0	0.6	0.3	0.3
Low fertilization with soluble green compost (LFSGC)	3.0		0		0	1.04	0.6	0.3	0.3

Table 3 Total (shoot) biomass weight and biometric parameters for *Hibiscus* grown in substrates with different fertilization treatments: standard fertilization (SF), low fertilization (LF), and LF with added soluble digestate (LFSD) or soluble compost (LFSGC).

	Growth parameters				
	Total fresh weight ($g\ m^{-2}$)	Total dry weight ($g\ m^{-2}$)	Leaf area ($cm^2\ pt^{-1}$)	Plant height ($cm\ pt^{-1}$)	Plant volume ($cm^3\ pt^{-1}$)
SF	911 a	255 a	2543 a	41.5 a	44.2 a
LF	465 d	135 c	1459 c	33.5 b	18.5 b
LFSD	627 c	185 b	2268 b	41.5 a	41.7 a
LFSGC	772 b	246 a	2303 ab	40.5 a	45.4 a
	Growth indexes				
	Relative growth rate ($mg\ g^{-1}\ d^{-1}$)		Net assimilation rate ($g\ m^{-2}\ d^{-1}$)		Leaf area ratio ($m^2\ kg^{-1}$)
SF	28.4 a		6.8 a		4.2 bc
LF	23.5 c		5.3 b		4.5 ab
LFSD	25.9 b		5.3 b		4.9 a
LFSGC	28.1 a		7.0 a		4.0 c

Each value represents the mean of replicates (n = 3). Statistical significance assessed through one-way ANOVA. Different letters within each column indicate significant differences according to Duncan's multiple-range test (P = 0.05).

Table 4 Leaf mineral content for *Hibiscus* grown in substrates with different fertilization treatments: standard fertilization (SF), low fertilization (LF), and LF with added soluble digestate (LFSD) or soluble compost (LFSGC).

					Macronutrients (g kg^{-1})	
	N	P	K	Ca	Mg	S
SF	24.6 a	3.6 a	21.4 c	15.3 a	5.7 a	5.2 a
LF	21.4 b	2.6 c	16.7 d	15.5 a	5.8 a	2.8 c
LFSD	21.3 b	3.0 b	22.5 b	15.0 b	5.2 bc	4.0 b
LFSGC	23.0 ab	2.7 bc	23.0 a	13.8 c	4.9 c	2.7 c
					Micronutrients (mg kg^{-1})	
	Fe	Mn	B	Zn	Mo	Ni
SF	163 a	701 a	40.0 a	29.9 a	0.6 b	0.9 b
LF	134 a	800 a	55.7 a	38.5 a	1.0 a	0.7 b
LFSD	96 a	877 a	48.4 a	29.7 a	1.0 a	0.9 b
LFSGC	110 a	774 a	49.4 a	33.3 a	0.9 a	1.2 a
				Beneficial elements and non-nutrient heavy metals (mg kg^{-1})		
	Al	Si	Na	Co	Pb	Cr
SF	46.6 a	316.2 b	657 b	0.4 a	1.1 a	0.3 a
LF	34.7 b	310.8 b	574 b	0.5 a	0.9 a	0.3 a
LFSD	34.6 b	297.0 b	596 b	0.4 a	1.1 a	0.3 a
LFSGC	50.6 a	438.2 a	866 a	0.5 a	0.9 a	0.4 a

Each value in the table represents the mean of replicates (n = 3). Different letters within each column indicate significant differences according to Duncan's multiple-range test (P = 0.05).

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