Foliar applications of different plant biostimulants promote growth and fruit quality of strawberry plants grown under nutritional limitation

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Abstract

Plant biostimulants are a new emerging category of products especially for organic cultivation that target the promotion of plant growth also throughout the overcome of a stressful situation. The present study aimed to investigate the influence of biostimulants on vegetative growth, production and fruit quality of strawberry, grown in nutrient limitation conditions. The experiment was conducted in greenhouse conditions on strawberry plants cv. Elsanta. Repeated preharvest foliar applications of different biostimulants (humic acids, alfalfa protein hydrolysate, seaweed extract, hydrolysis of microalga and chitosan) were tested. Our results showed that plant growth was positively affected by some treatments. The number of leaves per plant was significantly increased in alfalfa- and chitosan-treated plants (2 leaves/plant more than control ones) at the end of the trial. Plants treated with alfalfa presented 16% higher chlorophyll content in leaves compared to control ones and the accumulated biomass per plant was enhanced in treated plants, though not significantly. Significant higher production levels were found in plants treated with chitosan (+20%). As far as fruit quality, treatments proved to significantly influence fruit weight and fruit firmness. In particular alfalfa treatment increased fruit weight, while chitosan-treated fruits had higher firmness and a tendency to lower soluble solids values compared to the control ones, indicating a possible ripening-delaying effect of this biostimulant product and therefore a probable interesting prolongation of postharvest shelf life of fruits. To conclude, this investigation provides a better understanding of how biostimulant applications might help plant growth in a stressful situation such as nutritional limitation and how these products can promote fruit quality. These biostimulant products may therefore represent a potentially interesting tool, especially within the framework of organic farming, to counteract the effects of limited nutritional support available for the crop.

Keywords: Strawberry, plant biostimulants, abiotic stress, foliar treatments, fruit quality

Introduction

Strawberry (*Fragaria* x *ananassa* Duch.) is considered an economically important fruit crop worldwide (Rohloff, 2011). In Italy, plants are cultivated in open field or under controlled conditions and cultural managements follow the guidelines for integrated (IPM) or organic production (Foschi et al., 2010). Growing practices influence the final quality of products and environment around us. Indeed, organic farming is able to offer food with high quality levels and with less environmental impact compared to conventional production system (Reganold et al., 2010). For instance, organically grown strawberries had a higher quality (anthocyanin levels, antioxidant activity) and environment (e.g. soil) was also positively influenced compared to IPM/conventional cultivation practices (Fernandes et al., 2012). Unfortunately, the main problem in organic production is related to lower yield due to some limiting factors such as nutrient limitation (de Ponti et al., 2012; Orsini et al., 2016).

Since no synthetic fertilizers are allowed in organic farming, a recent solution to overcome stressful situations (e.g. nutrient deficiency) might be represented by the use of plant

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biostimulants. According to the European Biostimulants Industry Council (EBIC, 2015) “Plant biostimulant is a material that contains substance(s) and/or microorganisms whose function, when applied to plants or the rhizosphere, is to stimulate natural processes to benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and/or crop quality, independent of its nutrient content”. Biostimulants can be classified into different categories. Some of them are humic and fulvic acids, protein hydrolysates and other N-containing compounds, seaweed extracts and botanicals, chitosan and other biopolymers (du Jardin, 2015). The aim of this study was to investigate the effect of repeated preharvest applications of different plant biostimulants on growth and quality of strawberry plants grown in a stressful situation (nutrient deficiency) and therefore indicate new potential solutions to improve nutrient availability to plants in an organic system.

Material and methods

The experiment was conducted in semi-controlled conditions at the Research Centre Laimburg in South Tirol. Strawberry cold stored tray plants cv. Elsanta were transplanted on 21st of April 2016, in containers (25 x 47 cm; 4 plants per pot) containing a mixture of natural clay and white peat as substrate. No standard fertilization and no pest control were applied to plants during the evaluation period. Strawberry plants were treated with the products summarized in Tab. 1. Biostimulant products were exclusively applied via foliar spraying. The first treatment was carried out in May at pre-flowering and further treatments followed every 7 days until harvest, for total of 6 treatments. Control plants were sprayed with water. All the plants were thoroughly sprayed until the run off point using a hand sprayer. The experimental setup was a completely randomized design with four replicates per treatment (each treatment consisting of 16 plants).

<u>Vegetative parameters</u>

Trend in vegetative growth was weekly followed (from full bloom to the end of harvest) considering the number of leaves per plant and chlorophyll content. The total number of healthy, fully expanded leaves per plant was counted. Two young leaves per strawberry plant were randomly evaluated at each time in order to measure the chlorophyll content with a portable chlorophyll meter (SPAD – 502, Konica Minolta, Tokyo, Japan). Biomass accumulation (roots, leaves, crown, stolons and shoots) was calculated as the difference between the final and initial dry weight of strawberry plants.

<u>Productive performance</u>

Starting at approximately from 60 to 90 DAT (days after transplanting), ripe fruits were harvested (twice a week). The average fruit weight (grams) and the fruit yield per plant (grams plant^{-1}) were determined.

<u>Fruit quality evaluation</u>

The fruit quality was assessed at fully-ripened stage. A randomly selected 4-fruits sample per plant was collected. After measuring the firmness (with penetrometer - 6mm in diameter), titratable acidity was determined with a titrator (TitroLine easy, SCHOTT, Mainz, Germany) by titrating strawberry pulp to pH 8.2 using 0.1 M NaOH. Total soluble solids were evaluated using a portable refractometer (PAL-1, ATAGO, Tokyo, Japan).

<u>Statistical analysis</u>

Data normality was examined with Shapiro-Wilk test and homogeneity of variance was confirmed using Flinger-Killeen’s test. Differences among treatments were assessed through the analysis of variance (ANOVA) followed by the Least Significance Differences (LSD) post hoc test ($P < 0.05$). Each of the values reported in the tables/figures results from the average of 4 replications (vegetative parameters) and 3 replications (productive and qualitative parameters). Statistical analyses were carried out in R v. 3.3.1. (R Development Core Team 2016).

Table 1: Details on the list of biostimulant compounds that were used in the experimental trial.

Treatments	Active ingredient
CON	Control (water)
HAL	Humic acids from Leonardite
APH	Alfalfa protein hydrolysate
SEA	Macroseaweed extract (*Ascophyllum nodosum*)
SPI	Hydrolysis of microalga (Spirulina spp.)
CHI	Chitosan

Results

Starting from a similar vegetative situation among plants (about five leaves per plant at full-bloom), results at the end of harvest (Tab. 2) indicated that plants treated with alfalfa and chitosan significantly increased the number of total leaves per plant (7 leaves per plant) compared to control plants (5 leaves per plant). Regarding the chlorophyll content (SPAD values), Tab. 2 illustrates a highest chlorophyll content (+16%) in alfalfa-treated leaves compared to control ones, though not significant. Foliar applications resulted in promoting dry matter per plant. The maximum biomass accumulation was obtained in plants sprayed with chitosan but this increase was not significant (Tab.2).

Table 2: Effect of biostimulant foliar treatments on strawberry plant growth (number of leaves/plant, SPAD values and accumulated dry biomass/plant) at the end of harvest.

Treatment	Number of leaves/plant	SPAD values	Accumulated biomass/plant (g)
CON	5,3 ± 0,4 c	41,3 ± 1,7	4,8 ± 3,7
HAL	6,3 ± 0,5 bc	44,8 ± 2,3	6,0 ± 2,7
APH	7,7 ± 0,5 a	48,1 ± 4,5	8,6 ± 2,0
SEA	6,0 ± 1,6 c	45,4 ± 4,8	5,8 ± 2,8
SPI	6,4 ± 0,7 bc	44,3 ± 3,4	8,8 ± 2,6
CHI	7,2 ± 0,3 a	43,0 ± 3,0	9,9 ± 2,8
Significance	**	ns	ns

Means (± S.D.) within the same column followed by the same letter, do not differ significantly according to LSD test at $p < 0.05$ (N=4). Anova significant differences: ***$P<0.001$; **$P<0.01$; *$P<0.05$; ns: not significant

Plants treated with chitosan significantly increased yield (by approximately 28%). No significant differences were observed among other treated plants compared to control ones (Figure 1).

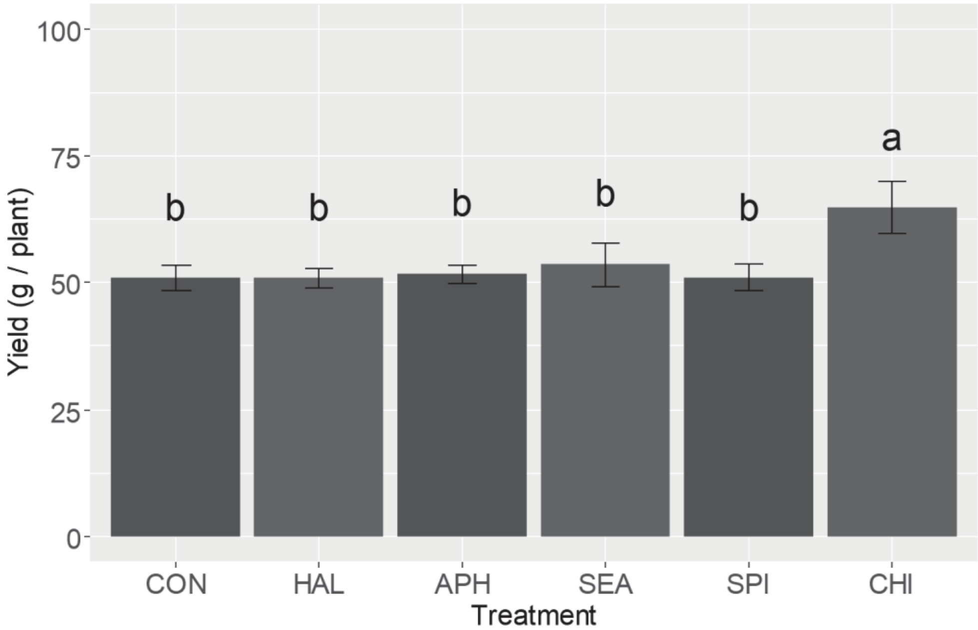

Figure 1: Effect of biostimulant foliar treatments on total yield per strawberry plant

Values are expressed as mean ± standard deviation. Different letters at the top of each bar indicate significant differences among treatments according to LSD test at p < 0.05 (N=3). Anova significant differences: **P<0.01.

Fruit quality data are reported in Tab. 3. Strawberries from the alfalfa treatment showed the greatest average fruit weight. A significant higher firmness and a tendency to lower soluble solids content values were observed in chitosan-treated fruits compared to the control ones. On the contrary, microalga-treated fruits had significantly lower firmness (–18%) and a tendency to higher titratable acidity (+12%) than non-treated fruits.

Table 3: Effect of biostimulant foliar treatments on strawberry fruit quality (mean fruit weight, firmness, total soluble solids TSS, titratable acidity TA) at harvest (red ripe stage).

Treatment	Mean fruit weight (g)			Firmness (kg/cm²)			TSS (°Brix)	TA (g/L)
CON	6,75	± 0,45	bc	0,71	± 0,02	b	7,07 ± 0,06	9,11 ± 0,09
HAL	6,59	± 0,41	bcd	0,69	± 0,02	b	7,73 ± 0,61	9,32 ± 0,08
APH	8,14	± 0,73	a	0,73	± 0,02	b	7,07 ± 0,51	9,48 ± 0,18
SEA	6,14	± 0,95	cd	0,72	± 0,01	b	7,73 ± 0,50	9,74 ± 0,19
SPI	5,52	± 0,39	d	0,58	± 0,09	c	7,03 ± 0,15	10,19 ± 0,99
CHI	7,56	± 0,84	ab	0,84	± 0,01	a	6,97 ± 0,15	8,72 ± 1,00
Significance	**			***			ns	ns

Means (± S.D.) within the same column followed by the same letter, do not differ significantly according to LSD test at p < 0.05 (N=3). Anova significant differences: ***P<0.001; **P<0.01; *P<0.05; ns: not significant

Discussion

Our data show that preharvest biostimulant foliar treatments influenced vegetative, productive and qualitative parameters on strawberry plants. Treatments with alfalfa and chitosan affected plant growth during the study period. This is partially in agreement with results reported by El-Miniawy et al. (2013), who showed that the number of leaves/plant was enhanced by 3 leaves after treatment with chitosan. On the contrary, no significant differences were detected as compared to control on the number of total leaves in plants treated with protein hydrolysate (Lisiecka et al., 2011). Alfalfa hydrolysate application increased leaf chlorophyll content as also shown in a previous study on tomato (Colla et al., 2014). According to Ertani et al. (2013), Mostafa (2015) and Mukta et al. (2017) alfalfa hydrolysate, seaweed extracts and chitosan applications on maize, fennel and strawberry, respectively, promoted plant growth, increasing the final biomass. However, no significant differences on accumulated biomass were recorded in our experiment. Data in Fig. 1 revel that total yield per plant (about 50 g/plant) was lower compared to a standard plant production, probably due to the fact that plants were grown under abiotic stress conditions (nutrient deficiency) without a standard fertilization during growing period. Although chitosan treatment proved to be a valid tool to increase yield per plant, this result should be confirmed with further investigations (e.g. in standard cultivation conditions). However, the increase in production as a result of treatments with chitosan is in agreement with that reported by Mukta et al. (2017) in strawberry. As far as fruit quality, fruit firmness was significantly higher in chitosan-treated fruits. A similar effect had been found by Bhaskara Reddy et al. (2000) and Hernández-Muñoz et al. (2008) on strawberry. On the contrary, the decreased firmness in fruits treated with microalga is in accordance with data reported by Masny et al. (2004). Macroseaweed extract has no significant effect on fruit quality in our experiment. Similarly, Spinelli et al. (2010) observed no changes in fruit quality in macroseaweed-treated fruits. In addition to greater firmness, chitosan-treated fruits showed also a tendency to decrease soluble solids in fruits, indicating a possible ripening-delaying effect of chitosan application and a consequent maintenance of quality during shelf life. Saavedra et al. (2016) also reported that chitosan application on strawberry fruits is an effective treatment to preserve storage life.

In conclusion, alfalfa hydrolysate and chitosan proved to be favorable treatments to promote plant growth and to improve fruit quality. More generally, the tested biostimulant products may therefore represent a potentially interesting tool, especially within the framework of organic farming, to help plants to overcome abiotic stress conditions, such as those related to nutritional limitation.

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