Effect of Biostimulants on Quality of Baby Leaf Lettuce Grown under Plastic Tunnel

A. Amanda and A. Ferrante
Dep. Produzione Vegetale
University of Milan
Italy

M. Valagussa
Mac - Minoprio Analisi e Certificazioni S.r.l.
Vertemate con Minoprio (CO)
Italy

A. Piaggesi
Valagro S.p.A. Piazzano di Atessa (Chieti)
Italy

Keywords: Actiwave, chlorophyll, environmental friendly, leafy vegetables, nitrate, yield

Abstract

Applications of biostimulants might be used for stimulating the nutrient use efficiency and improving quality of baby leaf vegetables. The aim of this work was to evaluate the use of biostimulant for reducing the nitrate content and improving the commercial quality. The field trials were performed under plastic tunnel in the North Italy vegetable production area (Bolgare, BG, Italy) during spring (April-May) and summer (July). The application of biostimulant (Actiwave®, Valagro S.p.a) was performed by spray using a modified fertigation machine. The concentrations of Actiwave applied were 0, 3, 4.5 or 6 ml m^{-2}. The effect of treatments was evaluated by the following determinations: yield, leaf nitrate content, total chlorophyll, total carotenoids, anthocyanins and total phenols content. In additional the health status of leaves was determined by chlorophyll *a* fluorescence measurements. Results obtained showed that the 4.5 and 6 ml m^{-2} Actiwave significantly increased the yield. The leaf nitrate content ranged between 900 and 1100 mg kg^{-1} FW and was slightly lower in the 3 ml m^{-2} treatment. The highest chlorophyll and carotenoids were found in lettuce treated with 6 ml m^{-2} Actiwave concentration. The anthocyanins and total phenols were slightly higher at higher concentration of bistimulant. Actiwave treatments did not affect the main chlorophyll *a* fluorescence parameters. This result might be due to an increase of light use efficiency in treated plants. In conclusion, the biostimulant was able to increase the yield, but slightly effect on nitrate metabolism was observed. The low leaf nitrate content in all treatments might be due to the optimal environment conditions.

INTRODUCTION

In the recent years, the use of biostimulants has been increasing and their application is becoming a common practice in the sustainable agriculture. Biostimulants are organic extracts that do not contain plant growth regulators, therefore the active molecules belong to amino acids and vitamins. The main effect of these products is to reduce fertilizers application and other chemical compounds in agriculture. The positive effect has to be referred to the stimulation of plant metabolism. In presence of adequate nutrients availability, biostimulants increase nutrient use efficiency and plant productivity (Russo and Berlyn, 1992). These proprieties are particularly important for leafy vegetables production, because their quality is also dependent by the leaf nitrate content that in some cases may limit the commercialization. The application of biostimulants directly in nutrient solution in closed growing systems such as floating system has been effective in reducing nitrate contents and improving quality (Vernieri et al., 2006). Recently in Italy the biostimulants are included in the national fertilizers regulation. Therefore their production and commercialization have been regulated and under government control. The cultivation of plants in soils highly fertilised and rich in organic matter content may give several problems for vegetables commercialization and for environmental safety. Leafy vegetables destined to minimally processed industry grown under plastic tunnels may have high nitrate content over the EU thresholds and appropriate management strategies must be applied. The use of biostimulants may give an additional freedom degree to growers for producing high quality leafy vegetables with low nitrate content.

The aim of this work was to study the effect of Actiwave on yield and quality of baby leaf lettuce grown under plastic tunnel in highly fertilised soils.

MATERIALS AND METHODS

Plant Materials

Lettuce (*Lactuca sativa* L. var. *acephala* tipo Batavia Rubia) was sown with density of 1.3 g m^{-2} under plastic tunnel in the North Italy vegetable production area (Bolgare, BG, Italy) during spring (April-May,) and summer (July) in the 2007. Manure distribution was performed 30 March 2006 and last soil disinfection with Vapam sodium was performed 15 April 2006. The fertilization applied by the grower in the first cycle was 3.3 N, 65 P, 87 K (mg m^{-2}) and 1.3 N, 0.83 P, 2.5 K (g m^{-2}) at the second cycle. The biostimulant (Actiwave®, Valagro S.p.a) was applied by spray using a modified distribution machine as top dressing distribution. Treatments were performed in two applications; in each distribution the following concentrations of Actiwave were delivered: 0, 1.5, 3, 4.5 ml m^{-2}. Distribution occurred in one week interval.

Sampling and Measurements

At beginning of experiments soil samples were randomly taken under the tunnel, for chemical analyses (Table 1). At the harvest time, yield, leaf chlorophyll, total carotenoids, phenols, anthocyanins and nitrate content were measured. Chlorophylls and carotenoids were extracted using methanol 99.9% as solvent. Absorbance readings of extracts were measured at 665.2, 652.4 and 470 nm. Total chlorophyll and carotenoids were calculated by Lichtenthaler's formula (1987).

Samples of the frozen tissue (100 mg) were ground in a pre-chilled mortar and extracted into methanolic HCl (1%). Samples were incubated overnight at 4°C in darkness. The concentration of anthocyanins was expressed in cyanidin-3-glucoside equivalents determined spectrophotometrically at 535 nm using an extinction coefficient of 29,600 (ε).

Phenolic compounds were extracted from 30-50 mg of leaves using 5 ml 1.2 M HCl in 99% methanol. Absorbance measurements were taken after overnight incubation at 4°C. Total phenolics were estimated by measuring absorbance at 320 nm using an UV-Vis spectrophotometer (Ke and Saltveit, 1989).

The fluorescence of chlorophyll *a* was determined on dark adapted leaves, randomly taken from the treatment plots and incubated for 40 min at room temperature. Chlorophyll *a* fluorescence (expressed in relative units) was measured using a portable Handy Plant Efficiency Analyser (PEA, Hansatech, UK). Leaf fluorescence was determined after illumination of 4 mm diameter leaf section (50.24 mm^2) with a light intensity of 3000 µmol m^{-2} s^{-1}.

Nitrate extraction was conducted in 30 ml icon glass using 100 mg of powdered dry sample and 10 ml of water as extracting medium. Icons were shacked for 2 h at room temperature and the suspensions were then centrifuged at 4000 rpm for 15 min. The clear extracts were used for analysis. Nitrate concentration was determined by the sulphanilamide-naphtylethylendiamine dihydrocloride method, after preliminary reduction of NO_3^- to NO_2^- by a copper-cadmium reductor column, according to the ISO 13395 procedures.

Statistical Analysis

Data were subjected to one-way analysis of variance and the differences among treatments were analyzed by Tukey's test ($P<0.05$). Each treatment was composed by 4 replicates.

RESULTS

Yield and Leaf Nitrate Content

The application of Actiwave increased the yield during spring (April-May). In average the treatment with 3 ml m^{-2} induced an increment of 200 g m^{-2} of fresh mass compared with control. The temperature recorded ranged from a minimum of 10°C to a maximum of 28°C. Higher concentrations did not give any additional benefit. In July, the positive effect of biostimulant was slightly observed at dose of 4.5 ml m^{-2} (Fig. 1A). In summer, the air temperature under the tunnel was higher than the optimal ones. The minimum during the night was 15°C and the maximum during the day was 35°C.

Leaf nitrate content was not statistically different among treatments, but lower content was found in the treatment with 3 ml m^{-2} in April-May and in 4.5 ml m^{-2} Actiwave in July. In average the nitrate content was 1000 mg kg^{-1} FW, in both periods.

Soil Nitrogen Content

The $N\text{-}NO_3$ and $N\text{-}NH_4$ contents in soil were not different among treatments in April-May. In July the $N\text{-}NO_3$ content in soil was not different among treatments, although lower content was observed in the treatments with 4.5 or 6 ml m^{-2} Actiwave (Fig. 1C). The $N\text{-}NH_4$ in soils, instead, was lower at higher concentrations of biostimulant in July (Fig. 1D).

Chlorophyll, Carotenoids, Phenols, Anthocyanins and Chlorophyll *a* Fluorescence

Total chlorophylls and carotenoids were higher at 4.5 ml m^{-2} Actiwave, while total phenols and anthocyanins increased at the highest concentration of Actiwave (Table 2). The chlorophyll *a* fluorescence was measured for evaluating an eventual stress induced by treatments. The Fv/Fm ratio is commonly used as stress marker in plant and did not change in control and treatments with value of 0.83-0.84 .The performance index (PI) did not statistically change among treatments. The reaction centres per cross section (RC/CSm) decreased by increasing the biostimulant concentrations (Table 3).

DISCUSSION AND CONCLUSIONS

Biostimulants are produced using different raw organic materials. The composition is often variable and difficult to standardize. Many active compounds are in traces and under the analytical instrumental detection and in plants may work synergistically. Therefore, studies on biostimulants application in agriculture have to be focused on their effect on plant physiology and metabolism rather than to try to identify the exact composition. This means to identify the targets of biomolecules and plant response.

Biostimulants are usually used in addition to common agriculture practice, for increasing yield, improving quality, enhance resistance against biotic and abiotic stresses. The organic biostimulants increased yield in many vegetables (Russo and Berlyn, 1992; Heckman, 1994; Vernieri et al., 2005) and improved quality in several ornamental plants (Poincelot, 1993). Our experiments showed that Actiwave was able to increase the yield. Analogous results were observed in rocket grown in floating system (Vernieri et al., 2005). Moreover, the moderate effect of Actiwave in reducing leaf nitrate content in lettuce might be explained considering that the leaf nitrate content was already low in control conditions. Therefore at physiological level an additional reduction may not be possible for cell homeostasis, in term of osmotic equilibrium. The low nitrate content in baby leaf lettuce should be due to the optimal environmental conditions (light intensity and temperature), especially during spring season. In floating system for growing leafy vegetables Actiwave was efficiently used for lowering the nitrate content in rocket at different nitrate concentration in the nutrient solution (Vernieri et al., 2005; 2006). The low nitrogen content in soil in the treatment with Actiwave can be associated to higher plant uptake. Biostimulants usually increase the roots efficiency and promote plant growth (Berlyn and Russo, 1990; Russo and Berlyn, 1992).

The chlorophyll content of treated lettuce increased at higher Actiwave concentration. The increase of green colour may have a positive effect on the vegetable market and consumer attractiveness. Since chlorophyll and carotenoids are tightly correlated, because the second have function protection against photo-oxidation and linearly increase with chlorophyll concentration. The effect of biostimulants on chlorophyll increase was also observed in woody plants such as English oak (Ferrini and Nicese, 2002). Total phenols and anthocyanins also increased in treated plants and along with carotenoids contribute to neutraceutical proprieties of lettuce.

The chlorophyll *a* fluorescence were used for investigating the effect of Actiwave on leaf functionality. The maximum quantum efficiency of photosystem II, Fv/Fm ratio, shows a high degree of correlation with the quantum yield of net photosynthesis and is a stress marker (Maxwell and Johnson, 2000). Results obtained demonstrated that Actiwave did not induce any toxic effect or stress conditions on plants. Analogous results were observed in birch treated with organic biostimulant (Richardson et al., 2004). On the contrary Actiwave may increase energy use efficiency reducing the active reaction centre per cross section.

In conclusion, these preliminary results suggest that Actiwave can be used for increasing yield and quality of leafy vegetables under optimal growing conditions. In fact, the positive effect of biostimulant can be appreciated when the plant metabolism works without environmental limitations.

ACKNOWLEDGEMENTS

The present work was funded by Sicily Region 2007-2009 Project AZORT "La concimazione azotata degli ortaggi: sviluppo e trasferimento di metodi innovativi per aumentare l'efficienza d'uso dei fertilizzanti, ridurre l'impatto ambientale e migliorare la qualità dei prodotti".

Literature Cited

Berlyn, G.P. and Russo, R.O. 1990. The use of organic biostimulants to promote root growth. Belowground Ecol. 2:12-13.

Ferrini, F. and Nicese, F.P. 2002. Response of English oak (*Quercus robur* L.) trees to biostimulants application in the urban environment. Journal of Arboriculture 28(2):70-75.

Heckman, J.R. 1994. Effect of an organic biostimulant on cabbage yield. J. Home and Consumer Hort. 1(1):111-113.

Ke, D. and Saltveit, M.E. 1989. Wound-induced ethylene production phenolic metabolism and susceptibility to russet spotting in Iceberg lettuce. Physiologia Plantarum 76:412-418.

Lichtenthaler, H.K. 1987. Chlorophylls and carotenoids: pigments of photosynthetic membranes. Methods in Enzymology. 148:350-382.

Maxwell, K. and Johnson, G.N. 2000. Chlorophyll fluorescence - a practical guide. J. Exp. Bot. 51:659-668.

Poincelot, R.P. 1993. The use of a commercial organic biostimulant for bedding plant-production. Journal of Sustainable Agriculture 3(2):99-110.

Richardson, A.D., Aikens, M., Berlyn, G.P. and Marshall, P. 2004. Drought stress and paper birch (*Betula papyrifera*) seedlings: effects of an organic biostimulant on plant health and stress tolerance, and detection of stress effects with instrument-based, non invasive methods. Journal of Arboriculture 30(1):52-61.

Russo, R.O. and Berlyn, G.P. 1992. Vitamin-humic-algal root biostimulant increases yield of green bean. HortScience 27(7):847.

Vernieri, P., Borghesi, E., Tognoni, F., Serra, G., Ferrante, A. and Piaggesi, A. 2006. Use of biostimulants for reducing nutrient solution concentration in floating system. Acta Hort. 718:477-484.

Vernieri, P., Borghesi, E., Ferrante, A. and Magnani, G. 2005. Application of biostimulants in floating system for improving rocket quality. Journal of Food, Agriculture and Environment 3(3&4):86-88.

Tables

Table 1. Soil proprieties of 0–30 cm depth measured in April before the beginning of experiments. Values are means (*n*=4).

Parameter	Units	Values
pH H_2O		7.8
pH KCl		7.2
Total limestone	g kg^{-1}	28.0
Active limestone	g kg^{-1}	11.0
Organic matter	g kg^{-1}	41.0
C	g kg^{-1}	24.0
Total N	g kg^{-1}	2.8
C/N ratio		8.6
N-NH_4^+	mg kg^{-1}	39.74
N-NO_3^-	mg kg^{-1}	28.79
P_2O_5 (Olsen)	mg kg^{-1}	341
K	mg kg^{-1}	78.2
Ca	meq $100g^{-1}$	14.62
Mg	meq $100g^{-1}$	2.11
Na	meq $100g^{-1}$	0.20
Cation Exchange Capacity (CEC)	meq $100g^{-1}$	17.13
EC (saturated mixture)	mS cm^{-1}	1.7

Table 2. Total chlorophyll, carotenoids, phenols and anthocyanins measured at harvest time in baby leaf lettuce grown under plastic tunnel (April-May) treated with 0, 3, 4.5 or 6 ml m^{-2} Actiwave. Values are means with standard errors (*n*=8).

Actiwave ml m^{-2}	Chl a+b Mg/g FW	Total Carotenoids mg/g FW	Total phenols ABS_{320nm} /g FW	Anthocyanins mg/Kg FW
0	0.49±0.032	37.75±0.316	1.34±0.062	78.27±3.968
3	0.47±0.022	35.67±0.398	1.21±0.063	74.92±4.699
4.5	0.52±0.033	40.06±0.443	1.28±0.087	78.68±4.130
6	0.48±0.020	39.51±0.192	1.38±0.045	83.58±3.068

Table 3. Fluorescence parameters measured at harvest time (April-May) in baby leaf lettuce grown under plastic tunnel and treated with 0, 3, 4.5 or 6 ml m^{-2}. Values are means with standard errors (*n*=16).

Actiwave ml m^{-2}	Fm (a.u.)	Fv/Fm (a.u.)	PI (a.u.)	RC/CSm (a.u.)
0	2617±54.3	0.84±0.003	1.42±0.106	825.5±27.32
3	2443±42.9	0.84±0.002	1.40±0.119	780.8±22.65
4.5	2497±50.8	0.83±0.003	1.46±0.099	792.0±19.67
6	2550±50.1	0.84±0.003	1.39±0.090	761.2±14.92

Figures

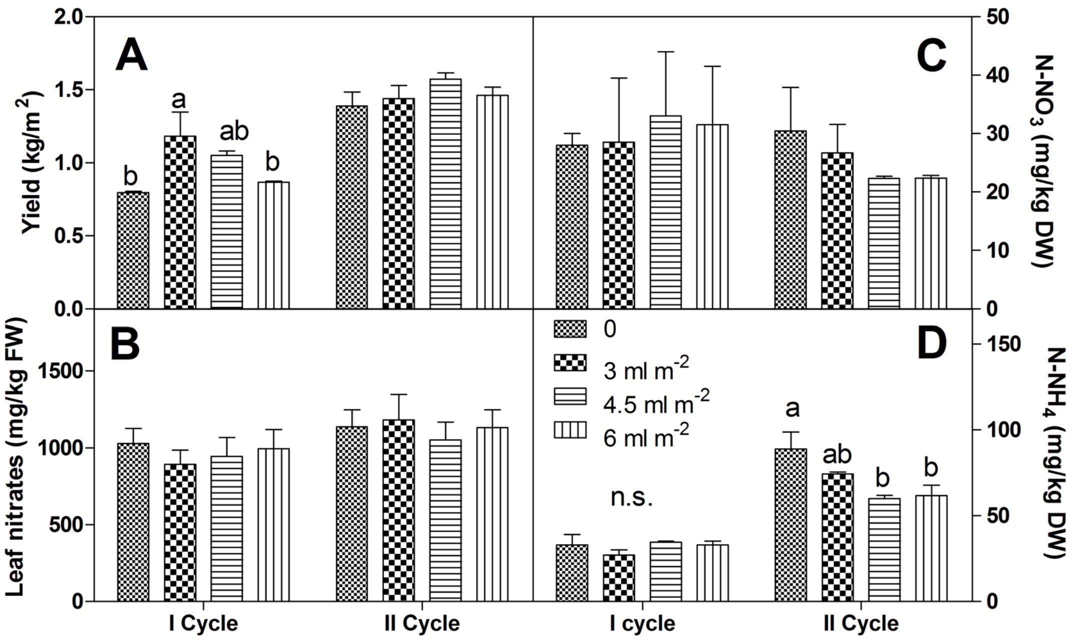

Fig. 1. Lettuce yield A), leaf nitrates B), $N\text{-}NO_3$ in soil, C) and $N\text{-}NH_4$ in soil, D) grown in April-May (I cycle) and July (II cycle) under plastic tunnels and treated with 0, 3, 4.5 and 6 ml m^{-2} Actiwave. Data was subjected to one way ANOVA analysis (*n*=4). Different letters indicate statistical differences at P<0.05.