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Shading and nitrogen management affect quality, safety and yield of greenhouse-grown leaf lettuce

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ABSTRACT

Two growing factors (light and nitrogen) supplied at optimal and sub-optimal levels were studied with the aim of evaluating their effects on greenhouse-grown lettuce' biomass and quality performances. According to a split-plot design with three replications, greenhouse lettuce was subjected to sixteen experimental treatments consisting of four photosynthetically active radiation (PAR) availability levels (0, 50, 65 and 85% PAR reduction) and four nitrogen fertilization rates (0, 75, 150 and 300 kg N ha^{-1}).

Response surface methodology (RSM) allowed to predict the highest achievable dry biomass (10.54 g plant^{-1}) at 0.9% PAR reduction and 185.4 kg N ha^{-1}. This optimal light/N combination induced a nitrate concentration of 1176 and 1826 mg kg^{-1} fresh weight (FW) in the inner and outer leaves, respectively. Shading decreased both the total phenolic content (TPC) and antioxidant activity. High N rates lowered both TPC in fully light condition and antioxidant activity in shading environment. The highest chlorophyll (Chl) concentrations were obtained with the combination shading/high N availability (at 85% PAR reduction and 300 kg N ha^{-1}) with values of 1.938 and 1.716 mg g^{-1} FW for Chla and Chlb, respectively.

In general, the results highlighted the potential for sustainable lettuce production, considering both economic and nutritional yields, i.e. providing high nutritionally dense products, slightly affecting the harvested biomass.

1. Introduction

Lettuce is a major crop grown in greenhouses, and it ranks high both in production and economic value among the vegetables grown worldwide (Křístková et al., 2008).

It constitutes an important functional food due to its content in vitamins, minerals and biologically active compounds (Kimura and Rodriguez-Amaya, 2003), such as photosynthetic pigments (chlorophylls) and phenolics. Such compounds play a major role in the prevention of various diseases associated with oxidative stress, functioning either as direct antioxidants or by augmenting the efficacy and production of other antioxidant molecules (Connor et al., 2005). Nevertheless, lettuce is also prone to accumulation of nitrates, which are recognized to be dangerous to human health due to several nitrate-induces syndromes, e.g. methemoglobinemia in infants (Fewtrell, 2004).

The accumulation of the abovementioned compounds, as well as its growth and yield, depend on environmental and management conditions (Liu et al., 2007), among which light and nitrogen play a preeminent role.

Light is a major factor affecting leaf traits and photosynthesis. When plants are exposed to low photosynthetic photon flux density (PPFD) avoidance syndromes, such as a decrease or increase of leaf blade area and petiole elongation, are observed; leaf development considerably differs in structure, photosynthetic pigments, electron carriers and photosynthetic rates compared to sunny leaves (Dai et al., 2009). Light supply also interact with the secondary metabolites production. The accumulation of flavonoids and phenols in herbs (Graham, 1998) is stimulated while the total phenolic compounds in *Beta vulgaris* var. conditiva Alef. storage roots (Stagnari et al., 2014) and in lettuce leaves (Galieni et al., 2015) are reduced as well as the anthocyanin concentrations in red lettuce (Kleinhenz et al., 2003). Radiation also acts through the enhancement of nitrate reductase activity, thus decreasing nitrate content in plants (Lillo,

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Abbreviations: ADF, acid detergent fiber; Car, carotenoids; Chl, chlorophyll; Chla, chlorophyll a; Chlb, chlorophyll b; DAT, days after transplanting; DM, dry matter; DW, dry weight; FW, fresh weight; GAE, gallic acid equivalents; IC, ion chromatography; LA, leaf area; NDF, neutral detergent fiber; PAR, photosynthetically active radiation; PPFD, photosynthetic photon flux density; RSM, response surface analysis methodology; SLA, specific leaf area; TAA, total antioxidant activity; TE, trolox equivalents; TPC, total polyphenols content.

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1994). Indeed, in lettuce nitrate concentration is negatively correlated with the content of soluble carbohydrates, which is associated with the stimulation of photosynthesis thanks to higher light levels.

Nitrogen is essential for normal lettuce growth and development, being an integral part of protein development and chloroplast structure (Barker and Bryson, 2007). Besides, excessive N availability leads to disease attack, detriment of leaf and root development (Mengel et al., 2001) and accumulation of nitrates. The latter results from an imbalance between nitrates uptake and their reduction to ammonia, and strongly depend on: N source (Abu-Rayyan et al., 2004); NH_4-N:NO_3-N N-fertilizer ratio (Stagnari et al., 2007); timing of N fertilizer release (Stagnari and Pisante, 2012); light intensity and duration (Gaudreau et al., 1995). Conversely, low N levels are reported to increase accumulation of phenolic compounds and the antioxidant capacity (Coria-Cayupan et al., 2009) as well as the concentration of ascorbic acid, flavonoids, and flavonols in *Arabidopsis* and tomato (Kandlbinder et al., 2004; Stewart et al., 2001).

To date, much research is based on maximizing horticultural production efficiency under different environmental conditions and agricultural practices. Recently, increasing interest has been focused toward the enhancement of the nutritional and health-promoting values of vegetables. Since the accumulation of secondary compound is often associated with yield decreases, the manipulation of the relationship "microclimate-biomass-quality" could represent the base of a sustainable production system.

On this basis, our study considered the following information: (1) N and light availability impact the biomass production and quality traits of lettuce, especially in terms of health-promoting phytochemicals and nitrate accumulation; (2) the aforementioned conditions can be easily set in greenhouse cultivation by manipulating culture practices; and (3) few studies have previously investigated on the interaction between light and N supply, focusing only on crop yield. The objective of this study was, therefore, to evaluate both the separate and combinate effects of two growing factors (light and nitrogen), supplied at optimal and sub-optimal levels, on nutritional quality and biomass production of greenhouse-grown lettuce.

2. Materials and methods

2.1. Plant material, growth conditions and experimental design

Two experiments were carried out from October 15 to December 19 2012, at the greenhouse of Agronomy and Crop Sciences Research and Education Center, University of Teramo (42°53′N and 13°55′E, 15 m a.s.l.). The environmental conditions, starting from transplanting until one week before harvesting, were constantly monitored (Fig. A1) with sensors of temperature and humidity connected to a data logger (EM50 Data Collection System, Decagon Devices Inc., Pullman, WA, USA).

Seeds of lettuce (*Lactuca sativa* L. cv. Bionda degli ortolani selection Siusi, FOUR-BLUMEN s.r.l., Piacenza, PC, Italy) were sown on a peat-based compost (peat:vermiculite:perilte at 1:1:1; v/v/v) with the following composition (percentage of dry matter): 40% organic carbon, 0.1% organic nitrogen, and 80% organic matter. Fifteen days after sowing, the seedlings (2 leaf stage) were transplanted into 15 cm × 15 cm plastic pots (2.5 l) at a density of 1 plant per pot. Pots were filled with sand, perlite and vermiculite at the ratio of 2:1:1.5 (v/v).

The experiment was arranged as a split-plot design with three replications, as follows: four shade conditions (main plots) and four nitrogen fertilization rates (subplots).

The shade treatments, 50, 65 and 85% reduction of photosynthetically active radiation (PAR, ranging from 400 to 700 nm) in addition to an unshaded control (0% reduction), were accomplished using different layers of shading nets provided by Agritenax S.p.A. (Eboli, SA, Italy). PAR intensity was measured with a PAR Photon Flux Sensor (Decagon Devices Inc., Pullman, WA, USA) connected to a data logger (EM50 Data Collection System, Decagon Devices Inc., Pullman, WA, USA) every hour from 10:00 a.m. to 16:00 p.m. The percent of shading was determined by comparing the daily average PAR values of nets with the daily average PAR values of the control treatment (greenhouse). Nets were wrapped around a rigid and removable structure (2.5 m × 2.0 m) placed above the vegetation, intercepting incoming light from the top and sides to 5 cm above the bottom of the pots. To allow air circulation, light was not limited from below.

The nitrogen fertilization rates were 0, 75, 150 and 300 kg N ha^{-1}. The established N levels were obtained by applying 0, 1.13, 2.25, and 4.50 g pot^{-1} of calcium nitrate ($Ca(NO_3)_2$) as nutrient solutions at 5 and 15 days after transplanting (DAT).

Each subplot consisted of 20 pots for a total of 80 completely randomized pots for each main plot (10 rows and 8 pots per row). To avoid edge effects, plants were daily re-randomized with the accuracy in maintaining the same sun orientation.

At transplanting plants were fertilized with simple superphosphate at 40 kg P_2O_5 ha^{-1}, potassium chloride at 100 kg K_2O ha^{-1}, and KSC Mix (Timac Agro Italia, Milano, Italy) at 0.02 kg ha^{-1}; KSC Mix is composed as follows: 15% water-soluble magnesium oxide (MgO); 28% water-soluble sulphurous anhydride (SO_3); 0.5% water-soluble boron (B); 0.5% water-soluble copper (Cu) chelated by EDTA; 2.5% water-soluble iron (Fe) chelated by EDTA; 2% water-soluble manganese (Mn) chelated by EDTA; 0.2% water-soluble molybdenum (Mo); 1.5% water-soluble zinc (Zn) chelated by EDTA. The pots were manually watered with tap water (pH 7.2 and EC of 0.23 mS cm^{-1}).

At 10 DAT a treatment with Ortiva fungicide (a.i. Azoxystrobin 23.2%, Syngenta Crop Protection S.p.A., Milano, Italy) at the dose of 0.08 ml m^{-2} was applied.

Growth and qualitative analysis were independently performed in both experiments.

2.2. Harvesting and leaf traits

All the lettuce plants were harvested at 50 DAT, when the unshaded plants had reached the stage of BBCH 43 (Feller et al., 1995). Ten plants for each subplot were selected, the roots were removed from the shoots and the leaves were counted and weighted collectively for fresh weight (FW) determination; leaves dry weight (DW) was obtained after drying in an oven at 80 °C for 72 h. Before drying, leaf area measurements to calculate the leaf area (LA; cm^2) and specific leaf area (SLA; cm^2 g^{-1}) were obtained by photocopying the leaves of each plant; the images were then acquired with an image analysis software (ImageJ, National Institutes of Health, Bethesda, MD, USA).

For each subplot, the leaves of the remaining plants were separated from roots, sub-sampled and immediately processed or flash-frozen, within 1 h of harvest, at −20 °C until being processed in the laboratory for analytical determinations performed on FW basis.

2.3. Chemicals and standards

All chemicals reagents and standards used for chemical analysis were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA).

2.4. Chlorophyll and carotenoid content

The concentrations of chlorophyll a (Chla), chlorophyll b (Chlb) and carotenoids (Car) were determined on fresh fully