ITALUS HORTUS
Original Research Report

Vol. 27 (2020), Pages 18-30
doi: 10.26353/j.itahort/2020.3.1830

Yield and quality of greenhouse multi-leaf lettuce cultivars grown in soil and soilless culture under Mediterranean conditions

Maria Gonnella, Massimiliano Renna[*] and Francesco Serio

Institute of Sciences of Food Production, CNR - National Research Council, Via G. Amendola, 122/O, I-70126 Bari, Italy
[*] Corresponding author: massimiliano.renna@ispa.cnr.it; Tel.: +39-080 5929306

Received: 11 November 2020; Accepted: 30 December 2020; Available online: 31 December 2020

Abstract: Multi-leaf lettuce has been proposed as a new type of product to be grown in open field or protected cultivation, especially for ready-to-eat salads. Like Batavia, oak leaf or lollo, multi-leaf lettuce is more attractive in characteristics such as size, colour, texture, but has smaller, more uniform leaves attached in a single point at the base. In the current research, we evaluated yield and quality of three multi-leaf lettuce cultivars (Ezra, Ezabel and Eztoril), in both autumn-winter and winter-spring cycles, by comparing soilless versus soil cultivation, and within soilless by comparing the supply of 50% nitrogen as ammonium form instead of sole nitrate fertilization. Soilless cultivation improved crop yield by about 20%, but only with the limiting environmental conditions of the first cycle. Among cultivars, Ezra always presented taller leaves than the others. Multi-leaf lettuce had good ammonium tolerance, never showing symptoms of toxicity. Ammonium nitrogen supply caused a slight increase in dry matter content, but only at the spring harvests. Simultaneously, it was responsible for a lower nitrate content, compared to nitrate-fed plants (-11 and -30%, respectively in first and second cycle). Generally, the nitrate content was quite low (2,470 and 1,000 mg kg^{-1} fresh weight, respectively in the two cycles), considering that it was a protected cultivation in winter or winter-spring. Under the operating conditions of our experiment, the influence of soil cultivation on dry matter and nitrate content was variable in relation to the cultivars. Mixed ammonium nutrition tended to increase the chlorophyll content in soilless-grown lettuce, only in the autumn-winter cycle, but this did not cause colour changes.

Keywords: Ezra; Ezabel; Eztoril; nitrate content; chlorophyll content; leaf colour; tunnel-greenhouse.

1. Introduction

Lettuce is the basic ingredient in fresh salads and ready-to-eat salad products. Lettuce types like iceberg, butterhead, romaine, Batavia, lollo and oak leaf provide enormous variety in shape, colour and flavour to minimally processed salad mixes. Like Batavia, oak leaf and lollo, the multi-leaf lettuce, proposed to growers as a new type, attracts consumers for its appearance characteristics such as size, colour, shape, texture, flavour (Martinez-Sanchez et al., 2012). Multi-leaf lettuce has leaves attached in a single point at the base, so a single cut releases all the leaves, smaller and more uniform than whole-head lettuce, but less susceptible to wound damage than baby leaf, due to less oxidation damage on reduced cut surface (Enza Zaden, 2020). Harvest can be done mechanically or manually in the field. Cutting plants below the growing point, a whole head can be obtained to be processed in the packaging area or sold to the end consumer for fresh consumption. In the preparation of salad mixes, multi-leaf lettuce offers the advantage of not having bits of leaf, deriving from cutting jagged edges of large blades or tiny leaves coming from the vegetative apex of the plant. These little bits are the first portion to rot, shortening the life of the whole salad bag. Compared with standard head lettuce, multi-leaf has greater efficiency (higher percentage of usable products, easier and faster processing); more attractive presentation in packaging (for three-dimensional leaves); minimal oxidation due to smaller stem diameter and better shelf life. One advantage of multi-leaf lettuce, when compared with baby-leaf, is the advanced maturity stage which confers higher crispiness (thicker and more resistant tissues to washing than baby leaves), and, as a consequence, a longer shelf life and then less waste during storage (Martinez-Sanchez et al., 2012).

Compared to both head lettuce and baby-leaf, multi-leaf lettuce is grown to an intermediate plant density (20-30 plants m^{-2}) and shows a compact plant habit. As regards fertilization, specific studies may be required in relation to its influence on product quality. Martinez-Sanchez et al. (2012) reported results about baby-leaf, multi-leaf and whole head lettuce post-harvest quality. Anyway, they did not mention the growing techniques and did not compare specific cultivars for the different types of raw materials. They used the same cultivars to obtain the three different raw materials, distinguished based on plant density and maturity stage at harvest. In their conditions, multi-leaf stage had higher dry matter content than baby-leaf and whole head, or only baby-leaf, according to cultivars (Martinez-Sanchez et al., 2012). Moreover, from the visual and microbiological point of view and for phytochemical content, baby-leaf had better quality (Martinez-Sanchez et al., 2012).

Raw material quality remains an essential prerequisite for the post-harvest quality, including textural quality and shelf life. The pre-harvest quality of lettuce depends mainly on the maintenance of a constant growth rate during the crop cycle, through the optimal management of nutrients and all the growth factors. Cultivation conditions, such as climate, culture system, irrigation, and fertilization, influence the quality of the raw material and can modify plant physiological behaviour and its suitability for fresh-cut processing (Hoque et al., 2010; Simonne et al., 2001; Fallovo et al., 2009; Scuderi et al., 2011; Luna et al., 2013). Greenhouse and soilless cultivation generally provide an optimized environment that results in a faster growth rate (Gruda, 2005). On the other hand, leaf texture, leaf dry matter content, colour brilliance and nitrate concentration are often negatively affected. Managing some environmental factors and nutrients, growers may enhance vegetable quality. In soilless grown baby-leaf lettuce and head lettuce, leaf dry matter content and firmness (Scuderi et al., 2011; Serio et al., 2001) were increased through a higher electrical conductivity of the nutrient solution. Other Authors studied the effect of different macroelements concentrations in the nutrient solution on tissue firmness and nitrate content in lettuce (Fallovo et al., 2009; Luna et al., 2013). Alternatively supplying a portion of nitrogen as ammonium in the nutrient solution may improve qualitative traits (such as dry matter content, colour intensity and chlorophyll content or nitrate content) in ammonium tolerant leafy vegetables. Although not so tolerant to ammonium as endive (which can be supplied with ammonium even as 100% of nitrogen) (Bonasia et al., 2008), lettuce, grown in a closed hydroponic system, was able to tolerate up to 30% of total N in the form of NH_4^+, showing an increase in fresh and dry shoot weight (Savvas et al., 2006).

Limited studies are available in the literature comparing the effects of soil and soilless systems on lettuce cultivars mainly addressed to fresh-cut produce. Pace et al. (2018) showed that soilless growing system can positively affect microbiological parameters (through a reduced increase of microbiological load during storage, compared to soil grown plants) and some quality traits of multi-leaf lettuce during 13 days of storage at 8 °C. Selma et al. (2012) studied different cultivars of lettuce (red oak leaf and lollo, and butterhead) grown on soil and soilless systems.

The aim of this study was to assess yield and quality of three multi-leaf lettuce cultivars by comparing soilless versus soil cultivation, and within soilless by comparing the supply of 50% nitrogen as ammonium form instead of sole nitrate fertilization.

2. Materials and Methods

2.1. Plant material and growing conditions

Lettuce (*Lactuca sativa* L.) plants of three multi-leaf cultivars (Ezra, Ezabel and Eztoril, respectively, red, light green and dark green from Enza Zaden Italia, Tarquinia (VT), Italy) were grown under soil and soilless systems in an unheated metal and plastic tunnel-greenhouse at the Experimental Farm "La Noria" of the Institute of the Sciences of Food Production of the National Research Council (Mola di Bari, Bari, 41°03' N; 17°04' E; 24 m a.s.l.).

Figure 1. Multi-leaf lettuce heads at harvest. A, Ezra; B, Ezabel; C, Eztoril.

Two growing cycles were carried out with transplanting on 29 October 2012 and 22 February 2013 for the autumn-winter winter-spring cycles, respectively. A split-plot design with three replications was applied, randomizing the growing systems (soil – S – and soilless system – SS – with nutrient solution containing the NO_3:NH_4 ratio 100:0 – SS100, and soilless with nutrient solution (NS) containing the NO_3:NH_4 50:50 – SS50) in the main plots and cultivars in the subplots. Main plots were of 3.6 m^2 (0.9 m wide and 4 m long). The soilless system consisted of three single trough-benches (4 m long × 0.3 m wide × 0.1 m high, with a slope of 2%) each plot, containing a 3:1 (v:v) perlite:peat mixture as substrate. The NS was supplied to the soilless systems without recirculation and contained 10 mM N (as N-NO_3 exclusively or 5 mM N-NO_3 and 5 mM N-NH_4 in the mixed nitrogen nutrition), 5.1 mM K, 1.6 mM P, 1.7 mM Mg, 4.8 mM Ca, 0.8 mM S. The last two nutrients were supplied at 3.5 mM and 1.8 mM, respectively, in the SS50 nutrient solution. Micronutrients were supplied according to Johnson et al. (1957). The irrigation water had the following composition (in mM): 0.3 NO_3-N, 0.2 K, 1.7 Mg, 1.8 Ca, 7.4 Cl, 4.0 Na. The electrical conductivity (EC) of the NS was 2.3 dS m^{-1} and the pH was 6.5.

The soil had the following characteristics: 24.3% sand, 31.9% silt and 43.8% clay, classified as clay soil (USDA Textural Soil Classification, 1987), pH 7.59, 1.4% organic matter, 0.95‰ total N, 220 mg kg^{-1} $BaCl_2$-TEA (barium chloride-triethanolamine)-extractable K, 98 mg kg^{-1} Olsen P, 1.13% total $CaCO_3$. At transplant S-plots were fertigated with ammonium nitrate and monopotassium phosphate giving the equivalent of 50, 80, 50 kg ha^{-1} of N, P_2O_5 and K_2O, respectively. Ten days after the first harvest, an integration of 30 kg ha^{-1} of N from ammonium nitrate was applied to the S-plots. Overall soil plots received a fertilization treatment similar to what usually applied to lettuce crop in the cultivation area.

Seedlings were produced in greenhouse in polystyrene trays on peat and were transplanted 25 d after sowing at the 4th true leaf stage.

Plants were placed at 0.15 m between rows and 0.13 m between plants in each row with a crop density of 51 plant m^{-2}. Temperature and relative humidity were passively controlled through ventilation. For the autumn-winter cycle, average daily air temperature was 13.6 °C; 2.0 to 16.1 °C minimum; 15.3 to 34.1 °C maximum. Average daily relative humidity was 90.8%. Photosynthetically active radiation (PAR) ranged from 12.7 to 194 μM m^{-2} s^{-1}. For the winter-spring cycle, average daily air temperature was 24.5 °C; - 0.2 to 17.6 °C minimum; 17.5 to 46.3 °C maximum. Average daily relative humidity was 50.5%. PAR ranged from 55 to 422 μM m^{-2} s^{-1}.

2.2. Plant sampling and measurements

During the growing cycles two harvests were done. The first harvest was performed 31 and 33 days after transplanting (DAT), respectively, in the autumn-winter and winter-spring cycle. In the first cycle, the cultivar Ezabel was harvested four days after the other two cultivars, while in the second cycle the soil treatment was harvested two weeks later than the soilless treatments. Harvest was carried out when plants reached at least 50 g weight, by cutting plants at 0.04 m from the collar in order to allow the plants to regrow and to have a second harvest (81 and 55 DAT, respectively in the first and second cycles). Fifteen plants were sampled per each treatment replication. Plants were immediately processed in laboratory for the planned measurements: plant fresh weight, height, colour leaf blade chlorophyll content (CHL a, b, total CHL) and total carotenoids content. An aliquot of plant fresh material was dehydrated to a constant weight in a thermo-ventilated oven at 65 °C for the determination of the dry weight and nitrate content.

Leaf colour was measured on five leaves per sample on the CIELAB scale 1976 (L, a^*, b^*) with a portable tristimulus colour-meter (Minolta Chroma Meter CR-400; Minolta Camera Co. Ltd.). The instrument runs with the colour-space coordinates designed as: L, the lightness value, ranging from black = 0 to white = 100; a^*, 'red/green chromaticity', red-violet colour if positive, green-blue colour if negative; b^*, 'yellow/blue chromaticity', yellow colour if positive, blue colour if negative. Through trigonometric functions, other colour indices were calculated: colour intensity or colour saturation, $C = [(a^*)^2 + (b^*)^2]^{1/2}$; and hue angle, $h° = \tan^{-1}(b^*/a^*)$, (where 0° = red-violet; 90° = yellow; 180° = blue-green; 270° = blue; McGuire, 1992).

Chlorophyll and total carotenoids contents were determined on leaf blade discs punched-out from five among the youngest fully-expanded leaves per sample. The pigments were extracted in 10 ml 80% acetone and the absorbance of the leaf extract was measured at 470, 645 and 662 nm using a UV-1800 Shimadzu spectrophotometer (Shimadzu, Kyoto, Japan). The amounts of chlorophyll and total carotenoids were estimated using the equation reported by Lichtenthaler and Welburn (1985), and expressed on a leaf weight basis.

Dry material was finely ground through a mill (IKA; Labortechnik, Staufen, Germany) with a 1.0 mm sieve, and used for quantitative chemical analyses. Inorganic NO_3^- was determined following the method described in Bonasia et al. (2008) by ion chromatography (Dionex DX120) with a conductivity detector, using a separation column IonPac AS14 and a pre-column IonPac AG14. Inorganic nitrate was extracted from 0.5 g of dried leaf tissue with 50 ml solution containing 3.5 mM sodium-carbonate and 1.0 mM sodium-bicarbonate through orbital shaking for 20 minutes at 140 rpm.

2.3. Statistical analysis

Statistical processing was carried out using the GLM (General Linear Model) procedure (SAS Software, Cary, NC, USA) by a strip-split-plot experimental design with the factor Harvest introduced as orthogonal factor in the split-plot experimental design applied in the greenhouse. The least significant difference (LSD) test ($\alpha = 0.05$) was used to establish differences between means.

3. Results

Table 1 shows means of the main factors of variability and the statistical results for biometric parameters and nitrate content.

In the autumn-winter cycle soilless cultivation produced on average 20% more than soil (Table 1) (especially in the second harvest; data not shown). The height of plants grown in the soilless system was higher than those grown in the soil (Table 1). Between the two soilless treatments the height of SS50 grown plants was higher than the SS100 plants. Cultivar differentiation was observed since cv. Ezra plant height was higher than the other two tested cultivars.

Table 1. Main effect of growing system, cultivar and harvest on yield, plant height, dry matter (DM) and nitrate content in two growing cycles (values for significant interactions are shown in Figures 2, 3 and 4).

Source of variation	Autumn-Winter cycle				Winter-Spring cycle			
	Yield (kg m^{-2})	Height (cm)	DM (g 100 g^{-1} fw)	Nitrate (mg kg^{-1} fw)	Yield (kg m^{-2})	Height (cm)	DM (g 100 g^{-1} fw)	Nitrate (mg kg^{-1} fw)
Growing system (GS)								
SS100	2.7	15.3 b	5.00	2655	2.6	13.5	6.22	1350
SS50	2.9	16.5 a	4.88	2353	3.1	15.5	5.31	946
S	2.3	14.5 c	4.88	2655	3.2	13.6	5.97	715
Cultivar (Cv)								
Ezra	2.5	19.3 a	5.08	2291	2.6	17.0	5.91	1192
Eztoril	2.8	14.7 b	4.91	2724	3.4	13.9	5.95	882
Ezabel	2.6	12.4 c	4.78	2395	2.9	11.6	5.65	938
Harvest (H)								
First	2.3	18.6	4.44	2545	2.5	13.9	5.50	1120
Second	3.0	12.3	5.40	2394	3.5	14.5	6.17	888
Significance								
GS	*	***	ns	ns	ns	ns	ns	*
Cv	ns	***	***	*	**	***	ns	*
H	ns	*	**	ns	*	ns	*	ns
GS×Cv	ns	ns	*	*	ns	ns	**	*
GS×H	*	ns	ns	*	ns	ns	ns	ns
Cv×H	ns	ns	**	*	**	***	ns	ns
GS×Cv×H	ns	ns	ns	ns	ns	ns	ns	ns

Significance: ns = not significant; * significant for $P \leq 0.05$; ** significant for $P \leq 0.01$; *** significant for $P \leq 0.001$. SS100 is for nutrient solution with NO_3:NH_4 100:0; SS50 is for NO_3:NH_4 50:50

The height of plants sampled at the first harvest was higher than those of the second harvest (Table 1). In the winter-spring cycle yield, not influenced by the growing system, resulted higher at the second harvest, specifically in the green cultivars, Eztoril and Ezabel (Figure 2). Plant height was higher on the second harvest as well, but not for the red cultivar Ezra, which inverted the response, being in addition distinctly taller than the other two cultivars (Figure 2).

Leaf dry matter content was on average almost 5 g 100 g^{-1} FW in the first cycle and only a small difference emerged between SS100 and S plants in Ezabel (Figure 3A). From the first to the second harvest, it increased, more markedly in Eztoril from 4.4 to 5.8 g 100 g^{-1} fw (data not shown). In the winter-spring cycle the response of cultivars to the growing systems was not univocal: while SS50 treatment gave higher DM content in plants of Eztoril and Ezabel, especially compared to SS100 plants, for cv. Ezra, plants grown in the soil showed the highest DM content (Figure 3B). In the first cycle, from the first to the second harvest, nitrate content showed a slight decrease in the green cultivars but not in the red one, which had a not significant increase (data not shown). In the autumn-winter cycle S-plants showed a lower nitrate content than SS100-plants for Ezra and Eztoril, while for Ezabel the lowest nitrate content was found in SS50-plants (Figure 3C). In the winter-spring cycle, in Ezra the nitrate content decreased from SS100 to SS50 to S-grown plants; for Eztoril SS50-plants showed a lower nitrate content than SS100-plants, while no difference was found in Ezabel among GS treatments (Figure 3D).

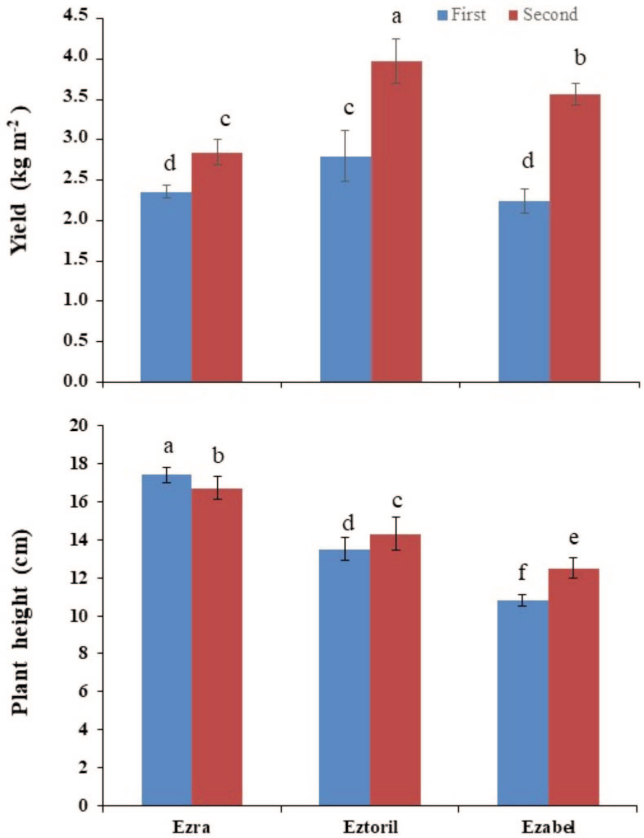

Figure 2. Effects of cultivar and harvest on yield and plant height (winter-spring cycle). Mean values (±SE) of three replicates and three growing systems.

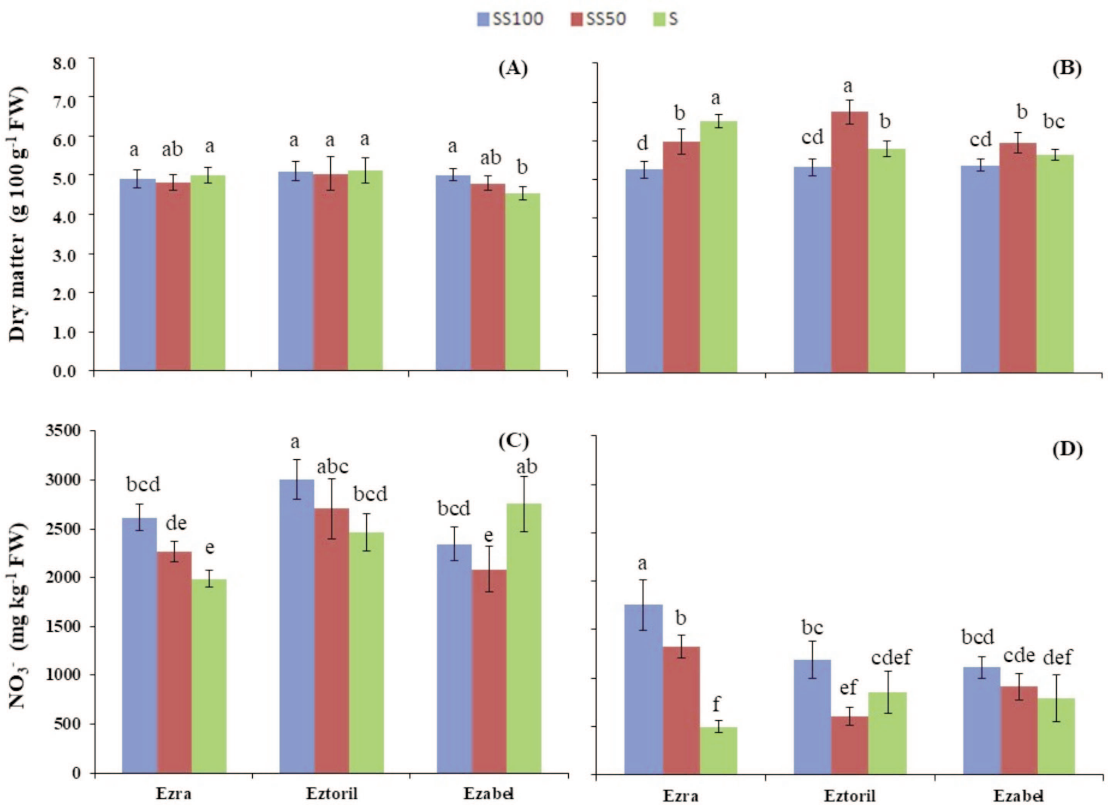

Figure 3. Effects of cultivar and growing system on dry matter and nitrate content: A and C, autumn-winter cycle; B and D, winter-spring cycle. Mean values (±SE) of three replicates and two harvest dates.

To this end, Figure 4 gives an overview of the nitrate accumulation during time. The decrease in nitrate content is clear from one cycle to the other (on average, 2,500 vs. 1,000 mg kg^{-1} FW), although not statistically compared. In addition, while in the first cycle nitrate content increased in SS100-plants by 19.1%, but decreased in SS50 and S-plants by 17.5% on average, in the winter-spring cycle a similar trend was observed but not statistically significant, with a mean decreasing content of nitrate from SS100 to SS50 to S-grown plants (Figure 4; Table 1).

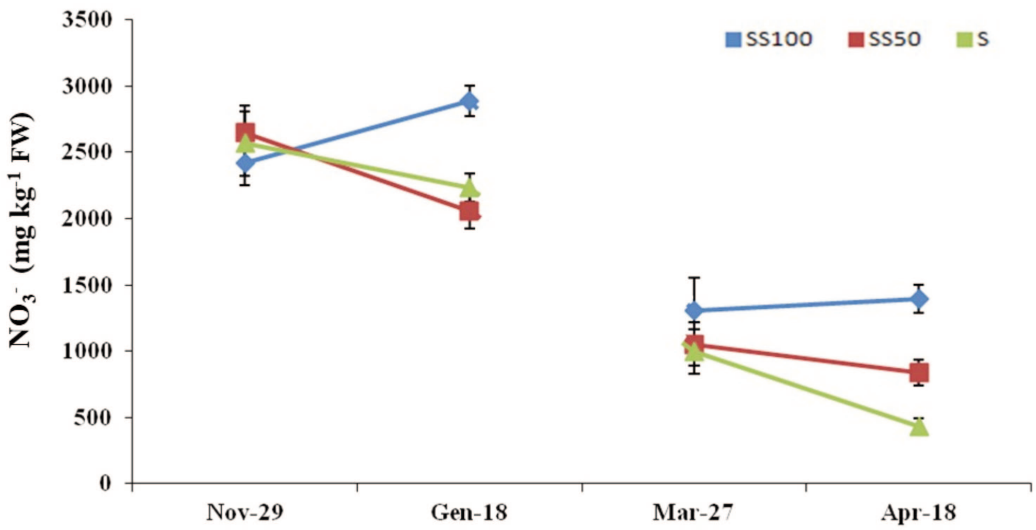

Figure 4. Effects of growing system and harvest on nitrate content: Nov-29 and Gen-18, autumn-winter cycle; Mar-27 and Apr-18, winter-spring cycle. Mean values (±SE) of three replicates and three cultivars.

Chlorophyll, total carotenoids content and leaf colour parameters are reported for the second harvest of both cycles (Tables 2 and 3). In the autumn-winter cycle SS50 plants showed a higher content of all chlorophyll a, b and total as well as total carotenoids (Table 2). Among cultivars, the red Ezra had the highest content of chlorophylls and carotenoids and the light green Ezabel the lowest ones (Table 2). As regards leaf colour parameters, Ezabel had the most luminous and saturated leaves, with a hue angle similar to the value measured on the dark green cultivar Eztoril (Table 2).

Table 2. Chlorophylls (Chl), total carotenoids content and leaf colour parameters in multileaf lettuce cultivars grown in the autumn-winter cycle at the second harvest (18/01/2013).

Source of variation	Chl a (mg g^{-1} fw)	Chl b (mg g^{-1} fw)	Total Chl (mg g^{-1} fw)	Total carotenoids (mg g^{-1} fw)	L*	C	h°
Growing system (GS)							
SS100	0.43 b	0.13 b	0.56 b	0.12 b	33.7	14.7 a	108.3
SS50	0.55 a	0.16 a	0.71 a	0.14 a	31.4	13.2 b	110.6
S	0.46 b	0.13 b	0.59 b	0.12 b	32.9	14.1 ab	106.4
Cultivar (Cv)							
Ezra	0.60 a	0.18 a	0.78 a	0.16 a	24.1 c	4.1 c	67.0 b
Eztoril	0.55 a	0.17 b	0.71 b	0.15 b	34.1 b	16.4 b	130.7 a
Ezabel	0.30 b	0.07 c	0.38 c	0.08 c	39.9 a	21.6 a	127.6 a
Significance							
GS	**	*	**	**	ns	*	ns
Cv	***	***	***	***	***	***	***
GS×Cv	ns	ns	ns	ns	ns	ns	ns

Significance: *** P ≤ 0.001; ** P ≤ 0.01; * P ≤ 0.05; ns, not significant. Mean values within each column followed by different letters are significant different at P = 0.05.

Table 3. Chlorophylls (Chl), total carotenoids content and colour parameters in multileaf lettuce cultivars in the winter-spring cycle at the second harvest (18/04/2013).

Source of variation	Chl a (mg g-1 fw)	Chl b (mg g-1 fw)	Total Chl (mg g-1 fw)	Total carotenoids (mg g-1 fw)	L*	C	h°
Growing system (GS)							
SS100	0.50	0.15	0.65	0.13	42.9 b	25.1	104.3
SS50	0.56	0.17	0.73	0.15	41.4 c	21.9	91.7
S	0.50	0.15	0.64	0.14	46.5 a	25.0	108.1
Cultivar (Cv)							
Ezra	0.51	0.16	0.67	0.15	31.4 c	8.1	73.2 b
Eztoril	0.68	0.21	0.88	0.17	47.3 b	31.0	122.3 a
Ezabel	0.36	0.11	0.47	0.09	54.1 a	35.9	118.8 a
Significance							
GS	ns	ns	ns	ns	*	ns	ns
Cv	***	***	***	***	***	***	***
GS×Cv	***	***	***	***	ns	**	ns

Significance: *** P ≤ 0.01; ** P ≤ 0.01; * P ≤ 0.05; ns, not significant. Mean values within each column followed by different letters are significant different at P = 0.05.

Results related to leaf colour parameters for cultivars were confirmed in the winter-spring cycle (Table 3), except that in this cycle a different response of the cultivars to the growing techniques was observed, in reference to the colour saturation C, since the red cultivar had a higher saturation value when grown in soil, compared to the soilless cultivation, while Ezabel showed a decreasing trend of C from soilless with nitrate nutrition to soilless with mixed nutrition to soil (data not shown). The lightness parameter L* changed between GS, showing the highest value in soil grown plants and the lowest in SS50-plants (Table 3).

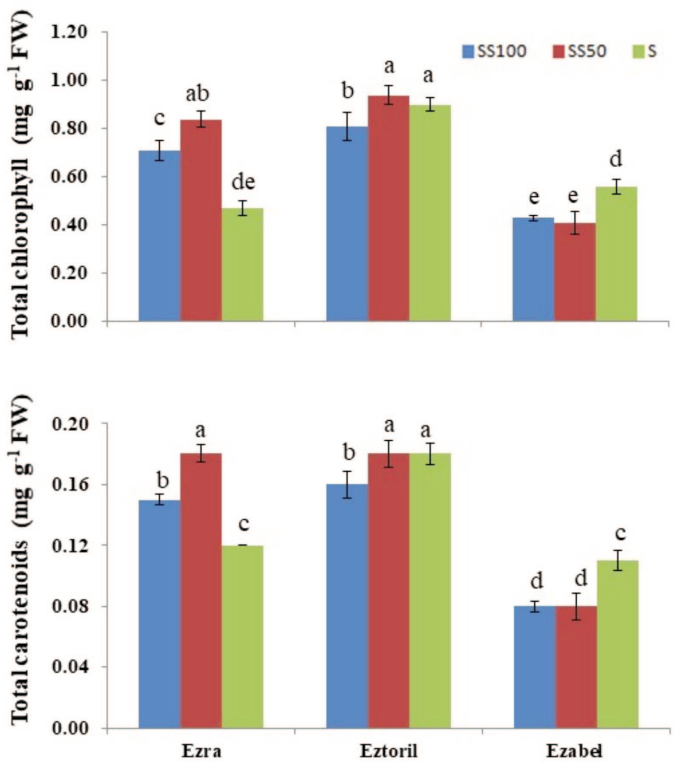

Figure 5. Effects of cultivar and growing system on total chlorophylls and carotenoids content (winter-spring cycle). Mean values (±SE) of three replicates and two harvest dates.

Regarding the pigment contents, a different response was observed in each cultivar in relation to the GS (Table 3; Figure 5). Specifically, in Ezra the highest and the lowest contents of chlorophylls and carotenoids were found in SS50 and S plants, respectively; for Ezabel the highest pigment content was observed in S plants; in Eztoril an intermediate response was observed, though SS100-plants showed the lowest content of total chlorophylls and carotenoids (Figure 5). From Figure 5 the lower content of pigments in the Ezabel cultivar, versus the other two, is clear and predictable since it is a light green lettuce. Chlorophyll a and b values for the significant interaction GS×Cv were not showed since they had a similar trend as total chlorophyll.

4. Discussion

Greenhouse lettuce cultivation can be strongly influenced by the microclimatic conditions. This is especially true if we are referring to a plastic greenhouse without climate control. The adaptation to the greenhouse environmental conditions determined the greatest differences in the morphological responses of the cultivars tested. As found in other studies (Marrou et al., 2013), the lower availability of light, especially in the first cycle, caused larger size (wider and longer leaves) and a horizontal orientation in plants than the specific habit of these cultivars, which typically produce short petioles and very closed and compact heads. From the first to the second growth cycle, yield did not change much for all cultivars tested, but the plants reduced the size and increased the dry matter content of leaves (Table 1), due to the faster growth determined by higher temperature and light (the second harvest was quite early in the winter-spring cycle, 55 DAT compared to 80 days of the first cycle). The fresh weight of plants at harvest was set at a minimum of 50 g per plant, referring to the minimum weight indicated for the cultivars studied. Harvest was made when weight was higher than the minimum, but the response of plants was different in relation to the cultivars and the growing cycle, especially in terms of height.

Soilless cultivation of multi-leaf lettuce cultivars improved crop performance as yield, particularly under limiting environmental conditions, that likely affect soil cultivation more than the soilless one. In fact, the yield differences between the two growing systems were marked during the autumn-winter cycle, when temperature and light were less favourable. In the next cycle, yield was similar in both systems (Table 1). We can hypothesize that the advantage of the soilless grown plants was due to the immediate and unlimited availability of nutrients in the soilless root medium, compared to the soil (Rouphael et al., 2004). This remains valid taking into account that lettuce increases nutrients uptake in the last weeks of the cycle, when plants experienced the most limiting conditions during the autumn-winter cycle. Two factors could act in this direction in our experiments. One is that plants grown in hydroponics require less energy to extract water and nutrients than plants in soil. Further in hydroponics a more precise control of nutrition and more efficient use of water and fertilizers is made possible by the limited volume of substrate per plant and a homogeneous composition (Raviv et al., 2019). The second point is that a better temperature regime is allowed by the higher water retention of the substrate, which lead to a greater thermal inertia than in soil (Rouphael et al., 2004).

Probably due to the unlimited availability of nutrients together with other favourable conditions (root medium conditions, absence of a nitrification inhibitor), a relatively high tolerance of lettuce to ammonium nutrition, supplied as 50% of the total nitrogen, occurred in the soilless grown plants. Actually, ammonium nutrition did not affect yield differently from nitrate nutrition (Table 1). On the other hand, ammonium supply changed markedly the nitrate content of lettuce (Table 1 and Figures 3 and 4). The results suggest that not only the supplied N ratio was not detrimental for yield and leaf appearance (colour, chlorosis and necrosis) in all lettuce cultivars, but also it had positive effect on reducing nitrate accumulation in leaves (by about 30 and 40% at the second harvest of the two growing cycles, respectively) (Figure 4). It was found that NH_4:NO_3 25:75 is the optimal ratio in the nutrient solution for lettuce growth in hydroponics or to be maintained in soil for both optimal yields and low nitrate content (Wang and Shen 2011). However, considering that in our study a nitrification inhibitor was not applied during the two growing cycles, it is reasonable that at least a part of the supplied ammonium was changed in nitrate in the root medium. This can explain the absence of toxicity symptoms in lettuce in our trials.

In addition to being influenced by the ammonium nutrition, nitrate was also modified by growing season, showing changes from the first to the second harvest (though statistically significant only in the first cycle) and from the first to the second experiment (Figure 4). This effect was clear in plants grown in soil and in those fed with ammonium, while in the nitrate-fed plants there was a discordant response, since it increased (first growing cycle) or remained unchanged (second cycle) in the second harvest compared to the first one (Figure 4). The behaviour observed in the first cycle can be due to the unfavourable interaction between high availability of nitrate ions in the root medium and low light exposition in the almost two months preceding the second harvest. This agrees with Buttaro et al. (2016) who observed a higher nitrate content in soilless cultivated rocket affected by low photosynthetic photon flux density. The nitrate content decreased from the first to the second growing cycle since plants were exposed to enhancing light conditions (Figure 4) as already observed in another study regarding several lettuce accessions (Burns et al., 2011).

In plants produced under cover, the red colour was concentrated in the outer leaves and apices of the inner leaves (data not shown). The same effect was observed in the colour intensity in the green cultivars, tough less remarkable. Red colour in lettuce is associated to anthocyanin content or total flavonoids (Gazula et al., 2007). The reduction of concentration of anthocyanins was observed in red lettuce cultivars due to the lower light intensity and higher temperature (Kleinhenz et al., 2003). These two factors, together with UV radiation, are the environmental conditions affecting lettuce colour in red cultivars grown under greenhouse. They generally act through changes in pigments concentration, specifically anthocyanins, but also chlorophylls a and b (Kleinhenz et al., 2003). Colour parameters can be useful to reveal pigments variation in lettuce products in relation to the environmental factors. They can change not only in relation to the environment of cultivation but also seasonally in the same environment. There was a moderate increase (about 30%) in leaf C and L* measured in each cultivar in the spring harvest compared to the winter one (Tables 2 and 3). The effect can be due to the higher light intensity. There was no change in hue angle level between the two cultivation cycles. Among leaf colour parameters, it is considered the one that best describes the colour variations in lettuce cultivars, since it can be positively correlated with low temperatures in the last weeks before harvest, as observed in red lettuce and with one of the phenolic compounds (caffeolylmalic acid CMA) (Marin et al., 2015). But generally speaking, it is not possible to establish a good correlation between hue angle and anthocyanins (Marin et al., 2015). On the contrary, chroma values were positively correlated with anthocyanins (Gazula et al., 2007). This was not one of the aims of our study but these considerations are useful since they suggest to deepen knowledge about the relationship between instrumental colour parameters and the content of pigments to study the optimal combinations of cultivation conditions and cultivars to enhance one of the most attractive characteristics of coloured lettuce.

Beyond the expected variation in colour and chlorophyll content due to very different cultivars, a marked effect of the cultivation system was observed for chlorophyll and carotenoid content (Tables 2 and 3). However, this effect showed opposite directions depending on cultivars. In fact, if the light green Ezabel had higher pigment content when grown in soil, the red lettuce Ezra had the lowest in soil, while the dark green Eztoril had slightly lower chlorophyll content if fertilized with nitrate nitrogen in soilless cultivation compared to soil and ammonium nutrition (Tables 2 and 3). All things considered, the expected effect of ammonium nutrition to increase green colour of leaves was not observed unequivocally, at the same way that such differences induced by soil cultivation versus soilless one did not gave the same results in all cultivars.

Finally, in both growing cycles only two subsequent cuts were carried out, with timing from each other affected by the cultivation season above all. It was expected that the winter-spring cycle would be shorter than the autumn-winter one, due to high light intensity, photoperiod and higher temperatures. The applied growing system and the studied cultivars allowed to extend the duration of the crop through repeated harvests and multiple cuts of leaves. This is a consistent economic advantage if fields are close to the processing farm in order to initiate the working process as soon as possible after harvest. The soilless cultivation allowed lettuce to mature two weeks before than soil in the winter-spring cycle. This can represent a further advantage, as already observed in other studies on soilless grown lettuce (Selma et al., 2012).

5. Conclusions

Under the operating conditions of our experiment, the yield and quality of the three lettuce cultivars were considerably influenced by the growing conditions and cultivation systems. However, the results differed between the cultivars. Soilless cultivation increased yield but only in the autumn-winter cycle, compared to soil. Ammonium nutrition did not alter yield but caused changes in dry matter content (only at the second cycle) and in nitrate content, generally decreased compared to the supply of nitrate, but not compared to soil. During the first growing cycle, ammonium nutrition increased the chlorophyll content, but this change did not correspond to an improvement in the colour of leaves. Finally, the cultivation technique applied to this type of lettuce allows subsequent cuts of leaves. In this study we only made two cuts, but there could potentially be more, depending on the environmental conditions and the sanitary status of plants. However, it is necessary that the product can be processed in a nearby factory, to preserve its qualitative traits until the start of the processing cycle for ready-to-eat production. Alternatively, plants can be harvested as whole heads for both processing and direct consumption after a full growth cycle.

Funding: This research was supported by MIUR (project 'High-convenience fruits and vegetables: new technologies for quality and new products', PON01_01435).

Acknowledgments: the authors wish to thank Dr. Giuseppe Cirillo for helpful contribution in the multileaf cultivation in open and protected environment, and Nicola Gentile for technical support during the experiments.

Conflicts of Interest: The authors declare no conflict of interest.

References

Bonasia, A., Conversa, G., Gonnella, M., Serio, F. and Santamaria, P. (2008) 'Effects of ammonium and nitrate nutrition on yield and quality in endive', *Journal of Horticultural Science and Biotechnology*, 83(1), pp. 64-70. doi: 10.1080/14620316.2008.11512348

Burns, I.G., Zhang, K., Turner, M.K., Meacham, M., Al-Redhiman, K., Lynn, J., Broadley, M.R., Hand, P. and Pink, D. (2011) 'Screening for genotype and environment effects on nitrate accumulation in 24 species of young lettuce', *Journal of the Science of Food and Agriculture*, 91(3), pp. 553-562. doi: 10.1002/jsfa.4220

Buttaro, D., Renna, M., Gerardi, C., Blando, F., Santamaria, P. and Serio, F. (2016) 'Soilless production of wild rocket as affected by greenhouse coverage with photovoltaic modules', *Acta Scientiarum Polonorum Hortorum Cultus*, 15(2), pp. 129-142.

Enza Zaden 2020 Eazyleaf: easy to slice lettuces. www.enzazaden.com/products-and-services/concepts-and-brands/eazyleaf (Accessed: 31/12/2020).

Fallovo, C., Rouphael, Y., Rea, E., Battistelli, A. and Colla, G. (2009) 'Nutrient solution concentration and growing season affect yield and quality of *Lactuca sativa* L. var. *acephala* in floating raft culture', *Journal of the Science of Food and Agriculture*, 89(10), pp. 1682-1689. doi: 10.1002/jsfa.3641

Gazula, A., Kleinhenz, M.D., Scheerens, J.C. and Ling, P.P. (2007) 'Anthocyanin levels in nine lettuce (Lactuca sativa) cultivars: Influence of planting date and relations among analytic, instrumented, and visual assessments of color', *HortScience*, 42(2), pp. 232-238. doi: 10.21273/hortsci.42.2.232

Gruda, N. (2005) 'Impact of environmental factors on product quality of greenhouse vegetables for fresh consumption', *Critical Reviews in Plant Sciences*, 24(3), pp. 227-247. doi: 10.1080/07352680591008628

Hoque, M.M., Ajwa, H., Othman, M., Smith, R. and Cahn, M. (2010), 'Yield and postharvest quality of lettuce in response to nitrogen, phosphorus, and potassium fertilizers', *HortScience*, 45(10), pp. 1539-1544. doi: 10.21273/hortsci.45.10.1539

Lichtenthaler, H.K. and Wellburn, A.R. (1985) 'Determination of Total Carotenoids and Chlorophylls A and B of Leaf in Different Solvents', *Biol. Soc. Trans.*, 11, pp. 591-592. doi: 10.1042/bst0110591

Luna, M.C., Martínez-Sánchez, A., Selma, M.V., Tudela, J.A., Baixauli, C. and Gil, M.I. (2013) 'Influence of nutrient solutions in an open-field soilless system on the quality characteristics and shelf life of fresh-cut red and green lettuces (Lactuca sativa L.) in different seasons', *Journal of the Science of Food and Agriculture*, 93(2), pp. 415-421. doi: 10.1002/jsfa.5777

Marin, A., Ferreres, F., Barbera, G.G. and Gil, M.I. (2015) 'Weather variability influences color and phenolic content of pigmented baby leaf lettuces throughout the season', *Journal of agricultural and food chemistry*, 63(6), pp. 1673-1681. doi: 10.1021/acs.jafc.5b00120

Marrou, H., Wéry, J., Dufour, L. and Dupraz, C. (2013) 'Productivity and radiation use efficiency of lettuces grown in the partial shade of photovoltaic panels', *European Journal of Agronomy*, 44, pp. 54-66. doi: 10.1016/j.eja.2012.08.003

Martínez-Sánchez, A., Luna, M.C., Selma, M.V., Tudela, J.A., Abad, J. and Gil, M.I. (2012) 'Baby-leaf and multi-leaf of green and red lettuces are suitable raw materials for the fresh-cut industry', *Postharvest Biology and Technology*, 63(1), pp. 1-10. doi: 10.1016/j.postharvbio.2011.07.010

McGuire, R.G. (1992) 'Reporting of objective colour measurement', *HortScience*, 27, pp. 1254-1255. doi: 10.21273/hortsci.27.12.1254

Pace, B., Capotorto, I., Gonnella, M., Baruzzi, F. and Cefola, M. (2018) 'Influence of soil and soilless agricultural growing system on postharvest quality of three ready-to-use multi-leaf lettuce cultivars', *Advances in Horticultural Science*, 32(3), pp. 353-362. doi: 10.13128/ahs-21927

Scuderi, D., Restuccia, C., Chisari, M., Barbagallo, R.N., Caggia, C. and Giuffrida, F. (2011) 'Salinity of nutrient solution influences the shelf-life of fresh-cut lettuce grown in floating system', *Postharvest Biology and Technology*, 59(2), pp. 132-137. doi: 10.1016/j.postharvbio.2010.08.016

Serio, F., Elia, A., Santamaria, P., Ruiz Rodriguez, G., Conversa, G. and Bianco, V.V. 2001. 'Lettuce growth, yield and nitrate content as affected by electrical conductivity of nutrient solution', *Acta Horticulturae*, 559, pp. 563-567. doi: 10.17660/ActaHortic.2001.559.82

Simonne, E., Simonne, A. and Wells, L. (2001) 'Nitrogen source affects crunchiness, but not lettuce yield', *Journal of Plant Nutrition*, 24(4-5), pp. 743-751. doi: 10.1081/PLN-100103667

Raviv, M, Lieth, J.H. and Bar-Tal, A. (2019) 'Significance of soilless culture in agriculture', in Raviv, M., Lieth, J.H. and Bar-Tal, A. (eds.), *Soilless culture: Theory and practice*. Elsevier Publications, pp. 3-14. doi: 10.1016/b978-0-444-63696-6.00001-3

Rouphael, Y., Colla, G., Battistelli, A., Moscatello, S., Proietti, S. and Rea, E. (2004) 'Yield, water requirement, nutrient uptake and fruit quality of zucchini squash grown in soil and closed soilless culture', *The Journal of Horticultural Science and Biotechnology*, *79*(3), pp. 423-430. doi: 10.1080/14620316.2004.11511784

Savvas, D., Passam, H.C., Olympios, C., Nasi, E., Moustaka, E., Mantzos, N. and Barouchas, P. (2006) 'Effects of ammonium nitrogen on lettuce grown on pumice in a closed hydroponic system', *HortScience*, 41(7), pp. 1667-1673. doi: 10.21273/hortsci.41.7.1667

Selma, M.V., Luna, M.C., Martínez-Sánchez, A., Tudela, J.A., Beltrán, D., Baixauli, C. and Gil, M.I. (2012) 'Sensory quality, bioactive constituents and microbiological quality of green and red fresh-cut lettuces (Lactuca sativa L.) are influenced by soil and soilless agricultural production systems', *Postharvest Biology and Technology*, 63(1), pp. 16-24. doi: 10.1016/j.postharvbio.2011.08.002

Wang, B. and Shen, Q. (2011) 'NH_4^+-N/NO_3^--N ratios on growth and NO_3^--N remobilization in root vacuoles and cytoplasm of lettuce genotypes', *Canadian journal of plant science*, 91(2), pp. 411-417. doi: 10.4141/CJPS10044

© 2020 by the authors. Licensee Italian Society for Horticultural Science (Società di Ortoflorofrutticoltura Italiana; SOI), Sesto Fiorentino (Firenze), Italy. This work is an open access article distributed under a Creative Commons Attribution-NonCommercial (CC BY NC) 4.0 International License (http://creativecommons.org/licenses/by-nc/4.0/).