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How to optimize the cultivation of arugula under elevated atmospheric CO_2 and different nitrogen forms?

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1. Introduction, Knowledge, Objectives

The leafy vegetable crop arugula offers a characteristic taste and contains several phytochemicals such as polyphenols, carotenoids and glucosinolates (Ramos-Bueno et al., 2016). The growing demand for arugula results in increasing areas under cultivation in Germany (Strohm et al., 2016). For these reasons breeders focus on developing new arugula varieties with high yields, good storability, good sensory characteristics and improved capacities to cope with abiotic and biotic stressors. Among the latter, downy mildew imposes the most significant threat to arugula. In the future, this may become even more important since abiotic factors such as increased atmospheric CO_2 concentrations may impose additional stresses to the plants. Moreover, there are efforts to optimize the application of fertilizer such as nitrogen (N) which is the most important element in plant nutrition. The use of ammonium-dominated N supply is a promising option as it lowers the nitrate content (Kim et al., 2006) and increases the glucosinolate content (Marino et al., 2016) of arugula leaves which provides health benefits and might protect the plants against biotic and abiotic stresses (compare Marino et al., 2016). Further, the predicted elevated CO_2 concentrations inhibit the assimilation of nitrate which can be overcome by application of ammonium-N (Bloom et al., 2010). Taken together, it appears to be the best choice to apply ammonium-dominated N fertilizer to arugula varieties with resistance towards downy mildew under future elevated CO_2 concentrations in order to produce healthy plants with high yields and good nutritional quality. However, there are no studies that examine the combinations of the three factors genotype, CO_2 concentration and N form on leafy vegetable crops. This experiment thus aimed at investigating the effect of ambient and elevated atmospheric CO_2 concentrations, two N forms (100 % nitrate-N and ammonium-dominated N) on two varieties of arugula with different sensitivities towards downy mildew, with emphasis on plant growth parameters.

2. Material and Methods

The experiment was conducted as a split plot design in climate chambers (HGC 0714, Weiss Technik UK Ltd., Königswinter, Germany). CO_2 supply was the main factor with arugula variety and N form being the sub-factors. Three sets of experiments were run: The first and second sets from 29 August till 12 October 2016, and the third set from 24 October till 6 December 2016. Each set consisted of one climate chamber set to 400 ppm CO_2 and one climate chamber set to 800 ppm CO_2.

Table 1. Definition of treatments.

Name	CO_2 supply (ppm)	Arugula variety	N form (Nitrate-N : Ammonium-N)
aTriNO	400	Tricia	100:0
aTriNH			25:75
aBeNO		Bellezia	100:0
aBeNH			25:75
eTriNO	800	Tricia	100:0
eTriNH			25:75
eBeNO		Bellezia	100:0
eBeNH			25:75

The *Eruca sativa* varieties chosen were `Tricia´ which is susceptible to infection with downy mildew, and `Bellezia´ which possesses intermediate resistance against this pathogen (both from Enza Zaden Deutschland GmbH & Co. KG, Dannstadt-Schauernheim, Germany). The plants were grown in clay substrate (Nullerde fein; Alpenflor Erdenwerke GmbH & Co. KG, Weilheim, Germany) with 30 seeds per pot. Eight pots per treatment were placed into each climate chamber (yielding 32 pots per climate chamber) with the following conditions: 20/18 °C (day/night), no light, 400 ppm CO_2. After one week, when the cotyledons were unfolded, the pots were arranged to their final position (distances of 11.5 cm x 14 cm). The light intensity at plant height was set to 1100 µmol m^{-2} s^{-1} and the daylength to 11 h, with dusk and dawn phases of one hour each. The relative humidity was 50 % and the air temperature 20 °C/18 °C (day/night). Water was applied by weighing each pot individually and giving a certain amount of water in order to substitute the water loss. Fertilizer was applied to yield 300 mg N, 185 mg P_2O_5 and 500 mg K_2O per pot. N was given as calcium nitrate in the 100 % NO_3^- treatment while the 75 % NH_4^+-25 % NO_3^- treatments received a mixture of 50 % ammonium sulphate and 50 % ammonium nitrate. “Ferty Basisdünger 1” (Planta Düngemittel GmbH, Regenstauf, Germany) was used to apply P and K. During the cultivation period, the diameter of the pots including the plants, and plant height were assessed for each pot separately once a week. At harvest (43-45 days after sowing, DAS), the appearance of healthy roots at the bottom of the pots was quantified by visual rating using a scheme ranging from 1 (very little) to 9 (very strong). The data were analyzed as split plot design (CO_2 as main factor, N form and arugula variety as sub-factors) with nested ANOVA followed by Tukey test using the R software package (R Core Team 2015). The following model was chosen: CO_2*N form*variety + error (climate chamber/(N form*variety)). The data on root appearance, as ordinal variables, were analyzed by Kruskal-Wallis test and Bonferroni *post hoc* test.

3. Results

The plant height was not affected by the CO_2 concentration and no interactions of the factors CO_2, arugula variety and N form were recorded. The variety `Tricia´ was significantly smaller than `Bellezia´ at 18 DAS but this difference disappeared later. At 32 and 39 DAS the impact of the N form became significant: Plants with 100 % nitrate-N were taller than those with 75 % ammonium-N and 25 % nitrate-N, this was already a tendency at 25 DAS (p=0.08). However, at harvest (43 DAS), no more differences between the experimental treatments were observed.

Table 2. Height of 30 arugula plants in a single pot. Treatments are explained in table 1. DAS = days after sowing. Averages of n=3. Different letters indicate statistically significant differences between the treatments at α=0.05 (ANOVA with Tukey test).

Treatment	Plant height (cm)				
	18 DAS	25 DAS	32 DAS	39 DAS	43 DAS
aBeNO	2.35 a	5.60 a	8.54 a	10.88 a	11.85 a
aTriNO	2.15 b	5.15 a	8.71 a	11.02 a	11.83 a
aBeNH	2.46 a	5.17 a	8.54 b	10.31 b	11.71 a
aTriNH	2.00 b	5.00 a	8.33 b	10.69 b	11.94 a
eBeNO	2.69 a	6.02 a	8.88 a	10.81 a	11.44 a
eTriNO	2.38 b	6.00 a	8.94 a	10.67 a	10.81 a
eBeNH	2.69 a	5.67 a	8.35 b	10.08 b	11.06 a
eTriNH	2.27 b	5.50 a	8.54 b	10.54 b	11.10 a

During the cultivation period, the diameter of the pot with 30 arugula plants was mainly impacted by N form and variety, and rather little by the CO_2 supply (except for 25 DAS, data not shown). Larger diameters were observed for the variety \`Tricia´ and for the plants supplied with 100 % nitrate-N, respectively (Fig. 1).

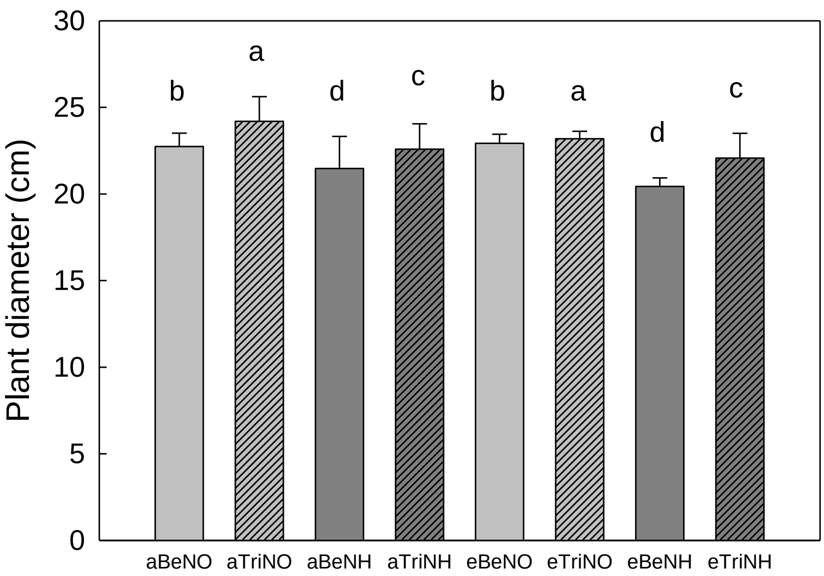

Fig. 1. Plant diameter (recorded as largest diameter of a pot with 30 arugula plants) at harvest (43 DAS). Treatments are explained in table 1. Averages of n=3+SD. Different letters indicate statistically significant differences between the treatments at α=0.05 (ANOVA with Tukey test).

Albeit less visible roots were found in the ammonium N-dominated pots, there were no significant differences among the experimental treatments (Fig. 2). Interestingly, each treatment by itself showed significant alterations of root appearance due to the CO_2 concentration.

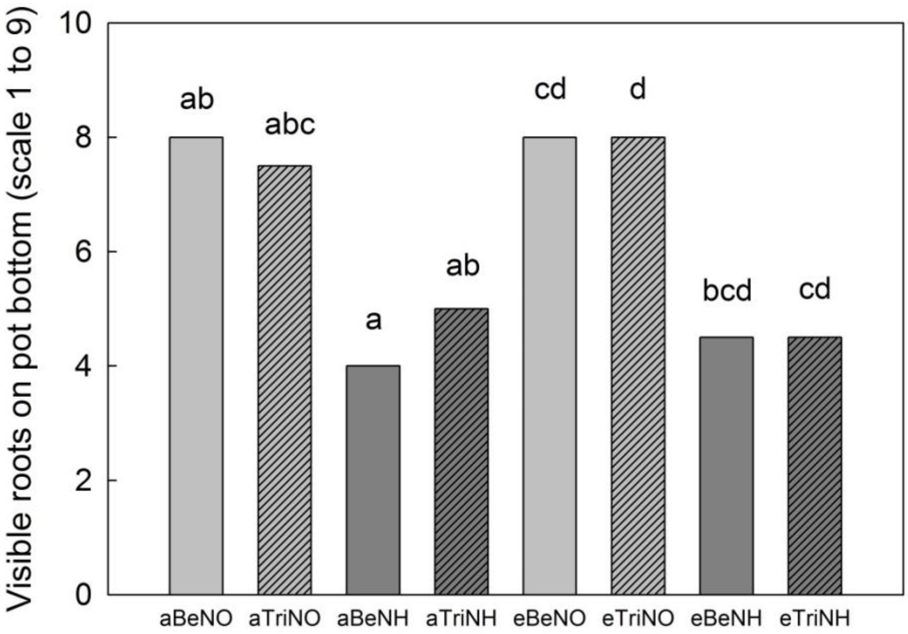

Fig. 2. Visual rating of visible healthy roots at the bottom of the pot at harvest (44-45 DAS). Treatments are explained in table 1. Medians of n=3. Different letters indicate statistically significant differences between the treatments at α=0.05 (Kruskal Wallis test with Bonferroni post hoc test).

4. Discussion

Little effects of the CO_2 concentration on plant growth parameters were observed which is surprising as it is known to result in increased yields of vegetable crops due to enhanced photosynthetic activity (e.g. Bisbis et al., 2018). Moreover, there were no interactions of the main factor CO_2 with the sub-factors arugula variety or N form, respectively. In fact, the arugula variety exerted significant impacts on plant growth parameters: \`Tricia´ pots had larger diameters which resulted in more fresh mass than \`Bellezia´ (data not shown). This is in accordance with Weinheimer & Naab (2014) who observed slightly lower yields for \`Bellezia´ as compared to \`Tricia´. However, the arugula variety \`Bellezia´ may be beneficial under conditions with high risks for infection by downy mildew.

Besides the arugula variety, the main impact on plant growth was imposed by the N form as plant receiving 100 % nitrate-N were higher, had larger diameters and more visible roots (Table 2, Fig. 1, Fig. 2). This was also reflected in the final aboveground fresh mass (data not shown). In contrast to our results, Kim et al. (2006) did not observe effects of 75 % ammonium-N compared to 100 % nitrate-N on plant height, leaf dry weight and root dry weight. We can confirm their results only for our plants at 43 days after sowing when they were no longer statistically different in height. However, Kim et al. (2006) applied the different N forms to another arugula variety in hydroponics only when the pants were already 28 days old, while our plants were grown in soil and received the different N forms for a longer time, which might have caused the different results.

Smaller root systems caused by ammonium-dominated N supply was reported for several other plant species, such as tomato (Ferreira Barreto et al., 2018) or barley (Britto et al., 2002) and are recognized as symptoms of ammonium toxicity (Britto et al., 2002; Esteban et al., 2016). In our study, besides the lower above- and belowground biomass, no toxicity symptoms caused by ammonium-N as described by Esteban et al. (2016) were observed.

5. Conclusions

The plant growth was mainly influenced by the N form, and to a lesser degree, by the arugula variety. Thus the choices of arugula variety and N form should be made according to the aims of the producers (high yield or high nutritional quality) and actual biotic and abiotic growing conditions (e.g. high pathogen pressure).

6. Literature

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