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The effect of reduced N fertilization on anthocyanins in red cabbage

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1. Introduction, Knowledge, Objectives

Red cabbage is not only important in human nutrition, but also increasingly interesting for dye production due to its content of anthocyanins. A high yield in anthocyanins could be achieved by agricultural practices such as irrigation or fertilization. Nitrogen (N) starvation yields in increased concentrations of anthocyanins in red cabbage (Hodges and Nozzolillo 1996). Lettuce plants show increased concentrations of anthocyanins upon N limitation (Bumgarner et al. 2012). However, no experiments were performed to date to examine the effects of reduced N supply on secondary metabolites of red cabbage. Thus, this study aimed at elucidating the impact of decreased N fertilization on the yield and the concentration of anthocyanins in the red cabbage `Lodero'. Moreover, we tested if the N concentration and the anthocyanin content of red cabbage can be determined in a non-invasive way by spectroscopic measurements.

2. Material and Methods

Red cabbage `Lodero' (Bejo Zaden B.V., Warmenhuizen, NL) was sown into pots in April 2012 and transferred to the field four weeks later. The plants were arranged on eight fully randomized plots (23.1 m^2 each) with 72 plants per plot. The amount of fertilizer was reduced by 50% in four plots (“Nred”) while the other four plots were fertilized regularly (“N”). This resulted in a one-factorial experiment with four replications.
For determination of fertilization requirements (198 kg N ha^{-1} in 0-90 cm soil depth for “N” plots, 99 kg N ha^{-1} in 0-90 cm soil depth for “Nred” plots), soil samples were taken in July 2012. The concentration of Nitrate-N was determined and based on these results, the plots were fertilized with calcium ammonium nitrate (Beiselen GmbH, Ulm, Germany). The plots were watered by irrigation sprinklers with calculation of the required amounts according to the Geisenheim irrigation scheduling (Paschold et al. 2002).
In October 2012, 50 plants from each experimental plot were harvested. The cabbage was peeled in order to determine the marketable yield. The non-marketable yield comprised plants that were elongated, infected by *Botrytis* or rotten, and cabbage heads which were too small to be marketable.
Three peeled marketable cabbage heads per plot were used for non-invasive spectroscopic measurements (Multiplex®, Force-A, France). One eighth of the upper part of the head was taken for analysis. Spectroscopic measurements were taken on three positions at the surface of this cabbage eighth. The parameters that reflect the N status were NBI_G and NBI_R and the anthocyanin content is indicated by FERARI, ANTH_RG and ANTH_RB (Tremblay et al. 2012). For validation by chemical analysis, the eighth parts

of the cabbage previously used for the spectroscopic measurements were cut into pieces and dried at 60°C (N) or frozen (anthocyanins). Each sample was ground and N was extracted according to the Kjeldahl method (Kjeldahl 1883) and determined by flow injection analysis. The anthocyanins were extracted from frozen material in 80% methanol with 0.1% acetic acid and their concentration was determined photometrically by absorbance readings at 500 nm and 700 nm (pH shift method, Wrolstad et al. 2005).
The data were compared by T test using the SigmaPlot 10.0 package (Systat Software, Inc., San Jose, CA, USA). For analysis of correlations between the spectroscopic parameters and the anthocyanin and N concentrations, regression analyses were performed (Sigmaplot 10.0).

3. Results

The marketable yield of red cabbage tended to be reduced by 4.6% in the “Nred” treatment compared to the "N" treatments ($p=0.06$, Fig. 1a). In contrast, there was no effect of the reduced N fertilization on the non-marketable yield per hectar. The proportion of marketable heads in relation to the total yield (59246.8 kg ha^{-1} for "N" and 57221.9 kg ha^{-1} for "Nred") did not differ between “Nred” and “N” treatments (Fig. 1a, data above the bar). The average mass per marketable head as well as its water content was not affected by reduced N fertilization (Fig. 1b).

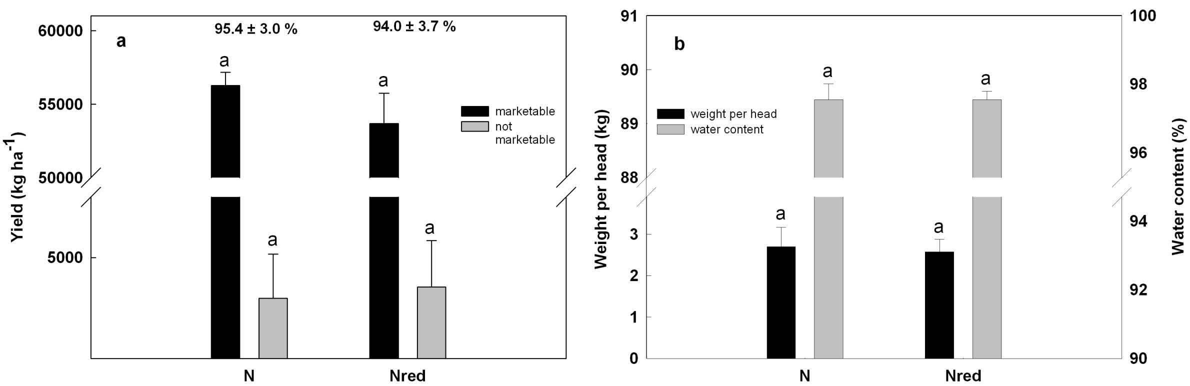

Fig. 1: Marketable and non-marketable yield (a), average weight per marketable head and water content (b) in red cabbage grown under normal and reduced N fertilization. In (a) the data above the bars show the average proportion of the marketable yield. N = 4 ± SD for marketable and non-marketable yield (a), N = 24 ± SD for average weight per head (b) and N = 24 ± SD for water content (b). Diff. letters indicate significant differences at $p = 0.05$.

The concentration of N in marketable heads was significantly reduced in the “Nred” treatment ($p<0.001$, Fig. 2a). In contrast, there was no effect of "Nred" on the concentration of total anthocyanins (Fig. 2b).
The spectroscopic parameters which represent the anthocyanin content of the sample (ANTH_RG, ANTH_RB and FERARI) were weakly correlated to the total anthocyanin content (Fig. 3a). The correlation of the N concentration with the spectroscopic parameters NBI_G and NBI_R was better (Fig. 3b).

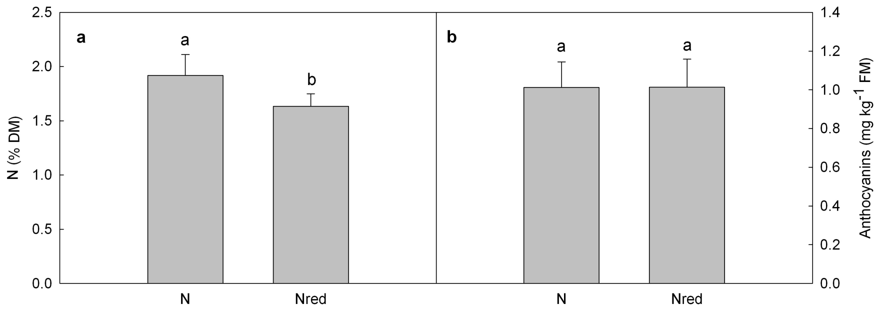

Fig. 2: Concentrations of N (a) and anthocyanins (b) determined by chemical analysis in marketable red cabbage heads grown with normal N and reduced N fertilization. N = 11-12 ± SD. Different letters indicate significant differences at p = 0.05.

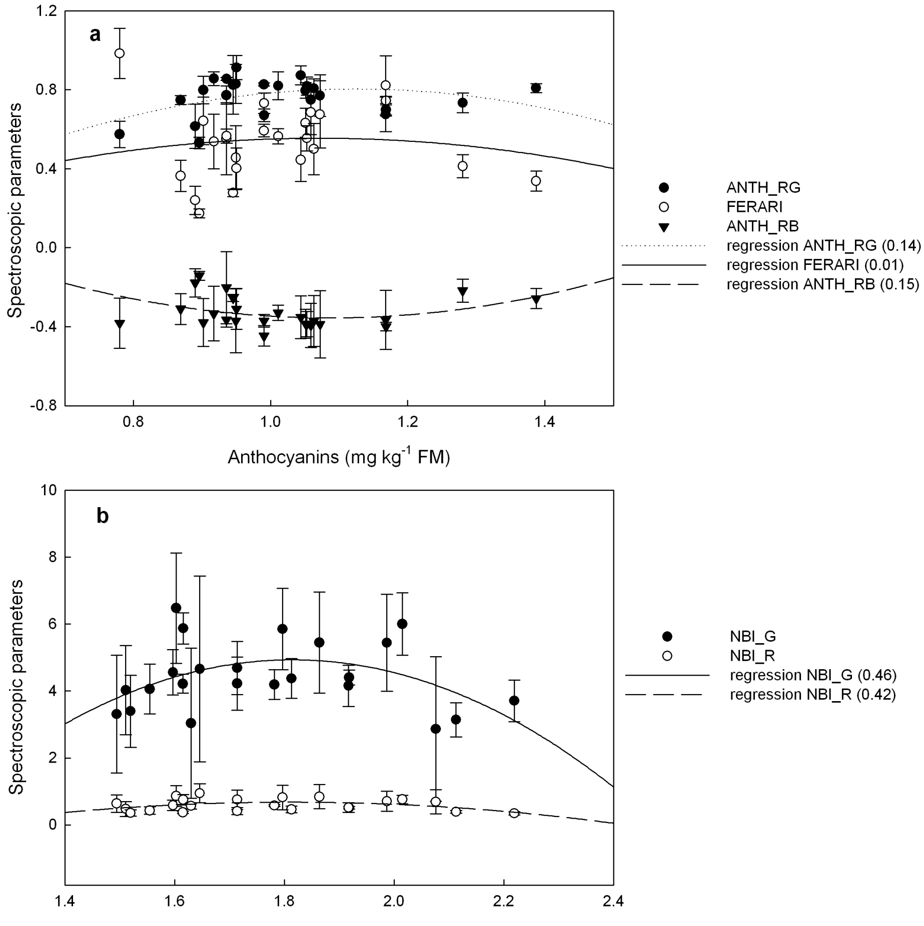

Fig. 3: Correlation of concentrations of anthocyanins (a) and N (b) with the respective spectroscopic parameters. Each curve was fitted by a polynomial quadratic function. In the legend, numbers behind the parameter´s names indicate the coefficient of determination (R^2) of the correlation with the anthocyanin or N concentration. The spectroscopic parameters are averages (N = 3 ± SD).

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4. Discussion

Influence of reduced N fertilization on yield and anthocyanin content

Reductions in N fertilization by 50% had no significant effects on yield of red cabbage as well as the proportion of marketable heads and the average weight per head (Fig. 1). These findings differ from those of Weier (1997) who reported yield reductions when N fertilizer was reduced by 20%. However, Weier (1997) investigated another variety of red cabbage which is probably more sensitive to N shortening than the variety `Lodero'.
When nitrogen is completely withheld in hydroponic culture, red cabbage shows inhibited growth as well as diminished chlorophyll content (Hodges and Nozzolillo 1996). The same plants had more anthocyanoplasts and higher anthocyanin content when compared to well-fertilized plants (Hodges and Nozzolillo 1996) which was not observed upon reduced N supply (Fig. 2b). The accumulation of secondary metabolites in response to N deprivation was reported for red beet as well (Salahas et al. 2011). Minimization of the primary metabolism, retardation of growth processes and accumulation of secondary metabolites are responses of plants when faced with stressful conditions. As the red cabbage plants in our experiments did not show significant reductions in biomass (Fig. 1) and no effects on anthocyanin content (Fig. 2b) although the N content was diminished (Fig. 2a), we conclude that the reduction of N fertilization by 50% is not a stressful factor for the red cabbage variety `Lodero'.

Correlation of spectroscopic parameters with chemical analyses

The correlations of the spectroscopic parameters that represent the anthocyanin and the N content with the respective chemically determined concentrations were very weak (Fig. 3). One reason for this may lie in the anatomy of red cabbage: The head consists of leaf layers that differ in thickness. In our experiment, only the outer leaf was measured while all layers were sampled. As the spectroscopic measurements performed on the outer layer of the head did not reflect its average content of N and anthocyanins, we assume that the chemical composition of each leaf layer varies. This needs to be investigated in further studies.

5. Conclusions

Our results suggest that in the cultivation of the red cabbage `Lodero' for dye production, the N fertilization can be reduced by 50% without significant losses of marketable yield as well as negative effects on the concentrations of anthocyanins.
The spectroscopic measurements by the Multiplex® device are not suitable for non-invasive determination of total N content as well as the concentration of anthocyanins in red cabbage heads.

6. Literature

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