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Yield and anthocyanin content in purple carrots with reduced nitrogen supply

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1. Introduction, Knowledge, Objectives

The demand for natural colouring substances, such as anthocyanins, is increasing. Purple carrots yield higher concentrations of anthocyanins than blueberries (Arscott & Tanumihardjo 2010) and are thus interesting as food colourants. For industrial production from purple carrots, high root yields and high concentrations of anthocyanins are requested. Depending on the cultivar, there is a strong variation in the anthocyanin content of purple carrot roots (Kammerer et al. 2004, Paschold et al. 2008). Little attention was paid to modifications of the anthocyanin content by agricultural practices such as fertilization. Several plant species showed increased concentrations of anthocyanins under limited nitrogen (N) supply (Hodges & Nozzolillo 1996, Lillo et al. 2008, Wang et al. 2012, Bumgarner et al. 2012, Ibrahim et al. 2012). No data are available on the impact of limited N supply on purple carrots. Thus, this study investigated whether it is possible to increase the anthocyanin concentrations in two varieties of purple carrots by reduced application of N fertilizer.

2. Material and Methods

The purple carrot varieties ´Deep Purple´ ("DP") and ´Purple Sun´ ("PS"; Bejo, Warmenhuizen, NL) were sown in the field in mid-June 2012. The experiment was conducted as two-factorial (factors: variety and N supply, table 1) block design with four replications. Water was supplied by drip irrigation according to the plants´ requirements. Upon appearance of the third leaf, the plots were fertilized with calcium ammonium nitrate (Beiselen GmbH, Ulm, Germany) to achieve 60 kg N ha$^{-1}$ in 0-30 cm soil depth ("SD"). The N levels were differentiated in early August 2012. N fertilizer was applied in order to achieve levels of 160 kg N ha$^{-1}$ in 0-60 cm SD, according to recommendations for industrial carrots (Feller et al. 2013) in eight plots ("con"). In another eight plots, the N levels were reduced by 50 % (= 80 kg N ha$^{-1}$ in 0-60 cm SD) as compared to recommendations ("Nred").
Table 1: Experimental design. Factors and number of plots (=replicates) per treatment.

<table>
<thead>
<tr>
<th>treatment</th>
<th>variety</th>
<th>N supply (kg ha⁻¹)</th>
<th>plots</th>
</tr>
</thead>
<tbody>
<tr>
<td>DP con</td>
<td>’Deep Purple’</td>
<td>160</td>
<td>4</td>
</tr>
<tr>
<td>DP Nred</td>
<td>’Deep Purple’</td>
<td>80</td>
<td>4</td>
</tr>
<tr>
<td>PS con</td>
<td>’Purple Sun’</td>
<td>160</td>
<td>4</td>
</tr>
<tr>
<td>PS Nred</td>
<td>’Purple Sun’</td>
<td>80</td>
<td>4</td>
</tr>
</tbody>
</table>

At harvest in late September 2012, the carrots were washed and classified into marketable and non-marketable (diameter less than 20 mm, splitted, broken, others) fractions. A subset of 5 plants per plot (= 20 plants per treatment) was weighed (fresh weight, FW), dried at 60 °C for 6 days and re-weighed for determination of dry weight (DW). The water content was calculated as ((FW-DW)/FW )*100. Another subset of 10 marketable plants per plot was randomly chosen for further measurements (length and weight of leaves and root, diameter of the root). Six of these roots were sampled for chemical analyses. The roots were cut and either dried at 60 °C or frozen in liquid N. Total N was analyzed from dry powder by elemental combustion (vario MAX CNS, Elementar Analysensysteme GmbH, Hanau, Germany). Total anthocyanins were extracted from ground frozen material in 80 % methanol and 0.1 % acetic acid. Their concentration was determined photometrically by absorbance readings at 500 nm and 700 nm according to the pH shift method (Wrolstad et al. 2005). The data were analyzed by Two Way ANOVA with variety and N supply as factors (PAST 2.15, Hammer et al. 2001). In order to further dissect differences between treatments, Mann Whitney pairwise comparisons were performed.

3. Results

 Marketable and non-marketable yield as well as the composition of the non-marketable fraction did not differ between the carrot varieties and N supply levels (Fig. 1).

![Fig. 1: Marketable (a) and non-marketable yield (b) for ’Deep Purple’ (DP) and ’Purple Sun’ (PS) with normal (con) and reduced (Nred) N supply. Different letters indicate significant differences between the treatments. N = 4 ± SD.](image)
DP had significantly longer roots and leaves than PS but the varieties did not differ in fresh mass (Fig. 2a). DP roots had larger diameters under optimal N supply (Fig. 2a). With reduced N supply, the root diameter decreased and was similar to PS (Fig. 2a). The leaf water content of PS was lower compared to DP for Nred-treated plants (Fig. 2b). No differences in the water content of the roots due to variety or N supply were observed.

Fig. 2: Biomass parameters (a) and water content (b) of leaves (L) and root (R) of ‘Deep Purple’ (DP) and ‘Purple Sun’ (PS) with normal (con) and reduced N (Nred) supply. Different letters indicate significant differences between the treatments. N = 39-40 ± SD.

The total N content in the roots did not differ between the carrot varieties (Fig. 3a). Reducing the N supply significantly reduced the total N and the total anthocyanins in roots of PS. With optimal N supply, DP roots had higher anthocyanin contents than PS (Fig. 3b).

Fig. 3: Total N (a) and anthocyanin content (b) of the roots of ‘Deep Purple’ (DP) and ‘Purple Sun’ (PS) with normal (con) and reduced N (Nred) supply. Different letters indicate significant differences between the treatments. N = 11-12 ± SD.
4. Discussion

DP had longer leaves and roots when compared to PS (Fig. 2a) but this did not impact the root weight. Thus, neither the marketable nor the non-marketable yields of the purple carrot roots were influenced by variety or N supply (Fig. 1). Under optimal fertilization with N the roots of DP had larger diameters compared to PS albeit this difference was marginal and did not reflect the root fresh weight (Fig. 2a). However, this was not the case when the plants were exposed to reduced N supply as the root diameter was significantly decreased in DP upon N limitation. The water content of the roots was not affected by variety or N supply level which suggests that both factors did not impact the dry matter in the roots. No comparable data are available for purple carrots. Different varieties of orange carrots showed no effects on yield (Westerveld et al. 2003, Heens 2013) or very low increases in dry matter when grown with reduced N supply (Seljasen et al. 2013 and references therein) which is similar to our observations.

The N content of the roots was not influenced by the carrot variety. Reducing the N supply resulted in significantly lower N concentrations in the roots of PS when compared to the controls. In the same way, the concentration of anthocyanins was increased upon N limitation in PS. PS contained more than 50 % less anthocyanins than DP when grown under optimal N supply (Fig. 3b). It seems to be possible to increase the anthocyanin content of PS to comparable levels as in DP when the N application is reduced by 50 % (Fig. 3b). However, these results should be interpreted with care as the missing difference between DP Nred and PS Nred might be due to the huge variation between the samples. The anthocyanin content of DP roots was in the order of magnitude reported by Leja et al. (2013). Reduction in N supply by 50 % had no effect on the anthocyanin content of this variety (Fig. 3c). Similar results were observed for the red cabbage variety `Lodero´ grown with reduced N fertilization (Schmidt & Zinkernagel 2013).

5. Conclusions

Under optimal N supply, the anthocyanin concentration is higher in `Deep Purple´ compared to `Purple Sun´ albeit both varieties have similar root yields. Consequently, `Deep Purple´ should be preferred if growing purple carrots for dye production. However, it is possible to increase the anthocyanin content in `Purple Sun´ but not in `Deep Purple´ by reducing the N supply.

6. Literature


Schmidt L, Zinkernagel J (2013) The effect of reduced N fertilization on anthocyanins in red cabbage. DGG-Proceedings. 3. 5 p. DOI: 10.5288/dgg-pr-03-06-ls-2013