Nitrogen Metabolism and Fresh Weight Production of Crambe under Different Nitrate Doses and Ph Values in Nutrient Solution

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Abstract: The aim of this work was to evaluate the effects of variation in nitrate (NO₃⁻-N) supply and different pH levels in nutrient solution on several aspects of nitrogen metabolism and the fresh weight production of crambe (Crambe abyssinica Hochst. ex. R.E. Fries). In the first experiment, crambe plants were submitted to crescent NO₃⁻-N doses (0.2; 2.0; and 4.0 mM) in hydroponic system located in a greenhouse. The second experiment was conducted with crambe plants cultivated under different pH values (5.0; 5.5; 6.0; and 6.5) also in nutrient solution at the same conditions. In both experiments the harvest was performed at the end of vegetative stage. Plants under pH 5.5 showed a high flux of NO₃⁻-N reduction and assimilation. The results obtained also indicate a key role of stem in NO₃⁻-N storage in crambe, probably as part of vacuolar pool. A significant increase in fresh weight of stem and leaves was obtained in plants submitted to 2 mM NO₃⁻-N and under pH 6.5. These results indicates a possible way to reduce the utilization of high NO₃⁻-N doses for crambe production and the necessity of soil adjustment, aiming the use of the culture for ruminant feed.

Key words: Crambe abyssinica Hochst. ex. R.E. Fries • Brassicaceae • Hydroponic system • Fresh weight • Nitrate reductase

INTRODUCTION

Crambe (Crambe abyssinica Hochst. ex. R.E. Fries) is an alohexaploid plant probably originated from Mediterranean that belongs to the Leptocrambe section of the Brassicaceae family [1, 2]. It is characterized as an annual and erect herb with approximately 0.8 m high and a cycle that lasts about 90 days [3, 4]. Crambe oil can be used in biodiesel industry, but is most commonly utilized for erucic acid (C22:1) isolation that is largely employed as lubricant, corrosion inhibitor or as raw material for synthetic rubber and plastic production [5, 6]. Furthermore, its cake have 30-32% of crude protein and is commonly used in ruminants feed [7]. However, despite the culture has a promising potential in different industries there is few information at metabolic level.

Nitrogen (N) is one of the most important nutritional factors in culture development. This is justified due to its requirement to vegetal metabolism, to represent high costs in productive process and to have the potential to cause serious impacts on environment, mainly by water table contamination [8, 9]. Other important factor for plant development is the pH of cultivation medium that can influence nutrient solubility and consequently its availability [10]. Furthermore, errors in pH adjustment can affect plants directly, since high concentration of H⁺ can damage roots tissues [11].

Thereby, information about metabolic behavior of crambe plants under variation in N supply or pH is needed to optimizing its production and sustainability. This information is a basic knowledge for commercial production of crambe, independently of which will be its final application.

Hydropony is a high utility tool for vegetal nutrition studies, since it allows the exclusion of the soil influence on ionic transfer process that occurs in root surface, making thus, the nutrient supply be provided in an almost constant tax. Furthermore, an important aspect of
hydroponic system is the easier manipulation of nutrients compared with the conventional method [12]. Another point to be considered about the use of nutrient solutions is the absence of some influence factors in metabolism, as soil-associated diseases and weeds [13]. Accordingly, the use of hydroponic system allows a more precise evaluation about interactions between nutrients and metabolic processes in plants.

Thus, the purpose of this work was evaluate the effects of crescent NO₃⁻-N doses and pH values in nutrient solution on several aspects of N metabolism in crambe plants to gather informations that contributes for the production of this culture with minimal use of inputs.

MATERIALS AND METHODS

General Informations and Seed Preparation: The experiments were conducted in hydroponic system at the greenhouse of Chemistry Department of Universidade Federal Rural do Rio de Janeiro during September–December 2012. In both experiments were used crambe (Crambe abyssinica Hochst. ex. R.E. Fries) seeds FMS brilhante variety previously treated under stirring with distilled water (15 min), 70% ethanol (1 min) and 2% sodium hypochlorite (15 min). After washing the seeds with distilled water, the sowing was performed using a sterilized substrate (“Mudas e plantio” - Biomix®) in a growth chamber.

Seedling Acclimation and Treatments: When the seedlings reached 10 cm, they were moved to the greenhouse for 7 days of acclimation with a 50% reduction in the light intensity. After this stage, the light reduction was abolished and the seedlings were transferred to 1.7 L pots in the hydroponic system. In both experiments, the hydroponic system was composed of pots connected to an air pumping machine and in each pot, one seedling was placed. The plants were cultivated for 1 week in a Hoagland and Arnon [14] nutrient solution modified with 2 mM NO₃⁻-N at ½ strength. Hereafter, in the first experiment the following NO₃⁻-N doses were applied: 0.2; 2.0; and 4.0 mM. In the second experiment, the treatments applied were pH 5.0; 5.5; 6.0; and 6.5. In both experiments a ½ strength Hoagland and Arnon [14] nutrient solution was used as a source of other nutrients and the nutrient solution was replaced weekly. Every 2 days, the pH was measured and adjusted and the solution volume was maintained by distilled water addition. At 68 days after germination (DAG) the harvest was performed.

Analyses, Experimental Design and Statistical: Roots, stems and leaves were separated and used for determination of fresh weight and 0.2 g samples were used for the analysis of nitrate reductase activity [15]. Furthermore, 0.5 g samples of each plant part were powdered in 20 mL of 80% ethanol, partitioned with chloroform and then used for amino-N [16], NO₃⁻-N [17], ammonium [18] and soluble sugar [19] analyses.

In both experiments, each treatment was composed of five replicates (plants) arranged in a completely randomized experimental design. An ANOVA was performed and the treatment means obtained were compared using Tukey’s test at the p = 0.05 level with Sigma Stat 3.2 software (Inc, Chicago, IL, USA).

RESULTS AND DISCUSSION

Fresh Weight Production: Table 1 shows that dose of 2 mM NO₃⁻-N caused significant increases in fresh weight production of stem and leaves. This result is important when considering that can be possible the cultivation of crambe for ruminant feed with this dose, avoiding additional costs and negative impacts on productivity related to the use of higher doses. An increase in fresh weight of stem and leaves also was observed in plants cultivated under pH 5.0 (Table 1). Since the NO₃⁻-N uptake is performed via 2 H⁺ symport, this result also showed that crambe does not have a high NO₃⁻-N dependency for the fresh weight production [20].

NO₃⁻-N Levels and Contents: NO₃⁻-N levels and contents in different parts of crambe plants showed a tendency to increase with the rise of its availability in nutrient solution (Table 2). Also as expected, plants cultivated under pH 5.0 showed high NO₃⁻-N levels and contents mainly in stem and leaves. These results confirmed that high H⁺ concentrations and NO₃⁻-N availability in nutrient solution favored the NO₃⁻-N uptake but not a significant increment in fresh weight parameters. Table 2 also shows that in both experiments crambe plants exhibit high NO₃⁻-N levels and contents in stem. This organ is not the preferential site for reduction reactions in most plant species which may indicate an important role of stem in the NO₃⁻-N storage [21]. In others members of Brassicaceae family like rape (Brassica campestris L.) and Chinese cabbage (Brassica chinensis), stem is the main site for NO₃⁻-N accumulation [22]. In preliminary studies was observed a tendency of the stem for NO₃⁻-N storage in wild-type crambe plants [4]. This mechanism occurs in many species aiming to maintain the N supply for biochemical activities in low availability periods [23, 24].
Table 1: Fresh weight (g plant⁻¹) in the roots, stems and leaves of crambe (Crambe abyssinica Hochst. ex. R.E. Fries) plants cultivated with different NO₃⁻N doses (0.2; 2.0; and 4.0 mM) and pH values (5.0; 5.5; 6.0 and 6.5 mM) in nutrient solution at the end of the vegetative stage.

<table>
<thead>
<tr>
<th>NO₃⁻N dose (mM)</th>
<th>pH value</th>
<th>Root</th>
<th>Stem</th>
<th>Leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>5.0</td>
<td>1.13a</td>
<td>2.26b</td>
<td>1.15c</td>
</tr>
<tr>
<td>2.0</td>
<td>5.5</td>
<td>1.55a</td>
<td>5.38a</td>
<td>4.45a</td>
</tr>
<tr>
<td>4.0</td>
<td>6.0</td>
<td>1.03a</td>
<td>3.58b</td>
<td>2.31b</td>
</tr>
<tr>
<td></td>
<td>6.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Different letters in the same plant part in each division (NO₃⁻N doses or pH values) indicate significant differences (Tukey’s test, p<0.05).

Table 2: NO₃⁻N levels (µmoles g⁻¹ FW) and contents (µmoles plant⁻¹) in the roots, stems and leaves of crambe (Crambe abyssinica Hochst. ex. R.E. Fries) plants cultivated with different NO₃⁻N doses (0.2; 2.0; and 4.0 mM) and pH values (5.0; 5.5; 6.0 and 6.5 mM) in nutrient solution at the end of the vegetative stage.

<table>
<thead>
<tr>
<th>NO₃⁻N level (µmoles g⁻¹ FW)</th>
<th>NO₃⁻N dose (mM)</th>
<th>pH value</th>
<th>Root</th>
<th>Stem</th>
<th>Leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>5.0</td>
<td>0.66b</td>
<td>0.49b</td>
<td>5.03a</td>
<td>18.98a</td>
</tr>
<tr>
<td>2.0</td>
<td>5.5</td>
<td>2.38c</td>
<td>12.37b</td>
<td>17.56a</td>
<td>47.4a</td>
</tr>
<tr>
<td>4.0</td>
<td>6.0</td>
<td>1.20c</td>
<td>5.08b</td>
<td>10.80a</td>
<td>29.26a</td>
</tr>
<tr>
<td></td>
<td>6.5</td>
<td>0.2</td>
<td>2.0</td>
<td>4.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

Note: Different letters in the same plant part in each division (NO₃⁻N doses or pH values) indicate significant differences (Tukey’s test, p<0.05).

Fig. 1: NO₃⁻N reductase activity (a) and NH₄⁺ levels (b) in roots, stems and leaves of crambe (Crambe abyssinica Hochst. ex. R.E. Fries) plants submitted to different NO₃⁻N doses in nutrient solution (0.2; 2.0; and 4.0 mM) harvested at the end of vegetative stage (68 days after germination). Different letters indicate significant difference in the same plant part (Tukey test, p=0.05).

Effects of NO₃⁻N in N Metabolism: Nitrate reductase activity showed a significant increase in leaves of plants under 4 mM NO₃⁻N compared with the plants under 0.2 mM of this ion (Fig. 1a). Plants exhibit commonly the nitrate reductase synthesis and activity higher in leaves and this enzyme is positive regulated by NO₃⁻N and indirectly by light [20]. Moreover, the low nitrate reductase activity in stem favored the hypothesis that this organ seems to be acting mainly for NO₃⁻N storage in crambe plants. The low enzyme activity and the high NO₃⁻N levels in stem could be due the presence of this ion in large amounts in vacuole that constitutes the substrate pool. This pool does not have a positive influence in nitrate reductase activity like the metabolic pool, formed by the NO₃⁻N that accumulates in citosol [25]. Ammonium levels showed a similar behavior in the different plant parts with a tendency to increase with the raise of NO₃⁻N supply (Fig. 1b). However, the increase of ammonium levels does not caused significant alterations and this enzyme is positive regulated by NO₃⁻N and in fresh weight production (Table 1). Plants submitted to 0.2 mM NO₃⁻N showed low ammonium and amino-N levels (Fig. 1b and 2a). This result is probably because after uptake NO₃⁻N can be reduced to ammonium and used to amino acids formation [21, 4].

The low soluble sugars levels in stem of plants under 0.2 mM NO₃⁻N can be also related to this result since the amino acids formation required carbon skeletons produced by soluble sugars degradation (Fig.2b).
Fig. 2: Amino-N (a) and soluble sugars levels (b) in roots, stems and leaves of crambe (Crambe abyssinica Hochst. ex. R.E. Fries) plants submitted to different NO$_3^-$-N doses in nutrient solution (0.2; 2.0; and 4.0 mM) harvested at the end of vegetative stage (68 days after germination). Different letters indicate significant difference in the same plant part (Tukey test, p= 0.05).

Fig. 3: NO$_3^-$-N reductase activity (a) and NH$_4^+$ levels (b) in roots, stems and leaves of crambe (Crambe abyssinica Hochst. ex. R.E. Fries) plants submitted to different pH in nutrient solution (5.0; 5.5; 6.0 and 6.5 mM) harvested at the end of vegetative stage (68 days after germination). Different letters indicate significant difference in the same plant part (Tukey test, p= 0.05).

Fig. 4: Amino-N (a) and soluble sugars levels (b) in roots, stems and leaves of crambe (Crambe abyssinica Hochst. ex. R.E. Fries) plants submitted to different pH in nutrient solution (5.0; 5.5; 6.0 and 6.5 mM) harvested at the end of vegetative stage (68 days after germination). Different letters indicate significant difference in the same plant part (Tukey test, p= 0.05).

Effects of pH in N Metabolism: A high nitrate reductase activity was detected in leaves of crambe plants under pH 5.5 and 6.0 (Fig. 3a). The increased nitrate reductase activity in leaves of plants under pH 5.5 probably caused the high ammonium levels of these plants (Fig. 3b). This effect is probably because of the importance of nitrate reductase activity in the metabolic pathway that leads to ammonium formation [22]. It is important to emphasize that perhaps brassicaceae is considered one of the ammonium sensible families, crambe plants does not show toxicity symptoms in this experiment [26].

High amino-N levels were observed in stem and leaves of plants under pH 5.5 (Fig. 4a). However Figure 4b shows that high soluble sugars levels were detected in plants under pH 6.0. These high levels can be linked to
the low use of carbon skeletons originated by soluble sugars degradation in amino acids formation because low amino-N levels were detected in these plants. Crambe plants under pH 5.5 showed high nitrate reductase activity and ammonium levels suggesting a major N flux to reduction and assimilation but not sufficient to cause a positive influence on fresh weight production.

CONCLUSIONS

Results indicate that crambe plants under pH 5.5 showed a high flow of NO\textsubscript{3}-N reduction and assimilation but with no positive effects on fresh weight production. Furthermore, the stem of crambe plants is the main site for NO\textsubscript{3}-N storage and probably with high percentage of this ion composing the vacuolar pool. Crambe plants under 2 mM NO\textsubscript{3}-N and pH 6.5 exhibit a significant increase in fresh weight production of stem and leaf. These results showed that is possible to avoid the use of high NO\textsubscript{3}-N doses in crambe cultivation and also the importance of pH adjustment of soil, especially considering the use of the culture in ruminant feed.

ACKNOWLEDGMENTS

The authors acknowledge the financial support provided by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), FAPERJ and CAPES. Technical support was provided by the Programa de Pós-Graduação em Química–UFRRJ.

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