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Influence of nitrogen fertilization on activities of ornithine transcarbamoylase and arginase in Chenin blanc berries at different stages of development

by

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Der Einfluß der Stickstoffdüngung auf die Aktivität der Ornithintranscarbamylase und der Arginase in den Beeren der Rebsorte Chenin blanc während verschiedener Stadien ihrer Entwicklung

Zusammenfassung. — Topfreben von Chenin blanc wurden mit vier Stickstoffkonzentrationen (0, 2, 4 und 8 mM NO_3^-) gedüngt; im Verlauf der Beerenentwicklung wurde in regelmäßigen Abständen die Aktivität der Ornithintranscarbamylase (OTC) und der Arginase in den Beeren bestimmt. Die OTC-Aktivität war am höchsten während des Weichwerdens der Beeren, nahm danach rasch ab und zeigte ein zweites kleineres Maximum zur Zeit der Beerenreife. Auch die Arginase wies im allgemeinen ihre höchste Aktivität während des Weichwerdens auf und nahm dann gegen die Beerenreife allmählich ab; eine Ausnahme bildete die Variante mit 8 mM NO_3^-, die einen Anstieg der Arginaseaktivität in den letzten 5 Wochen der Beerenreife zeigte. Mit ansteigenden Stickstoffgaben nahm die Aktivität sowohl von OTC als auch von Arginase im allgemeinen zu. Das Beerengewicht stieg bis zu 4 mM NO_3^- an; auf die Höhe des Mostgewichtes hatte die Stickstoffversorgung jedoch keinen erkennbaren Einfluß.

Introduction

The biosynthetic pathway for synthesis and degradation of arginine in *Vitis vinifera* L. was recently elucidated through enzymatic studies. The enzymes ornithine transcarbamoylase (OTC) (8), arginosuccinate (ASA) synthetase (9), arginosuccinate lyase (9), and arginase (10) were detected in extracts from grapevine leaves, mature fruits and seedlings and the optimum conditions for *in vitro* activities were determined.

Kliewer (6), Kliewer and Cook (7) and Bell *et al.* (1) have shown that the concentration of arginine in mature grape berries is positively correlated to the level of nitrogen fertilization of grapevines.

In the present study, the influence of different levels of nitrogen fertilization on activities of OTC and arginase in grape berries were determined at periodic intervals from shortly before veraison until fruit maturity. The influence of nitrogen on berry fresh weight and total soluble solids were also determined.

Materials and methods

5-year-old own-rooted *Vitis vinifera* L. cv. Chenin blanc vines were grown in 5-gal. metal cans containing a sterilized mixture of soil, sand and peat (2 : 2 : 1, v/v).

Each vine was pruned to two to three-bud spurs. Beginning shortly after budbreak, three shoots were trained vertically on separate 6-ft. stakes with the remaining shoots removed. The vines were irrigated with Hoagland No. 1 nutrient solution modified to contain 0, 2, 4 and 8 mM NO_3^- at weekly intervals and with tap water every second day or daily during periods of heat stress. There were 10 vines per treatment, each vine serving as a replicate in a randomized complete block design.

Grape berries were randomly sampled from each vine at periodic intervals, beginning about 1 week before veraison (Fig. 1). Berry samples from all replicate vines of a single treatment were composited, transferred to the laboratory, washed, blotted dry and weighed. 20—30 g berry samples were then randomly taken for enzyme extraction and total soluble solids determination.

OTC and arginase were extracted from berry tissue as described previously (Roubelakis and Kliewer 1977 a and c) except for the following modifications: the amount of grape tissue used for extraction was 20—30 g; 5 ml of grinding medium were used per g of fresh tissue; and the pH of the grinding and homogenizing media were 8.2 and 8.0, respectively. The reaction mixtures, reaction conditions and methods for determination of enzyme activities were the same as previously described (8, 10). In all cases, enzyme activity data are mean of three extractions per sampling date and three determinations of enzyme activity per extraction.

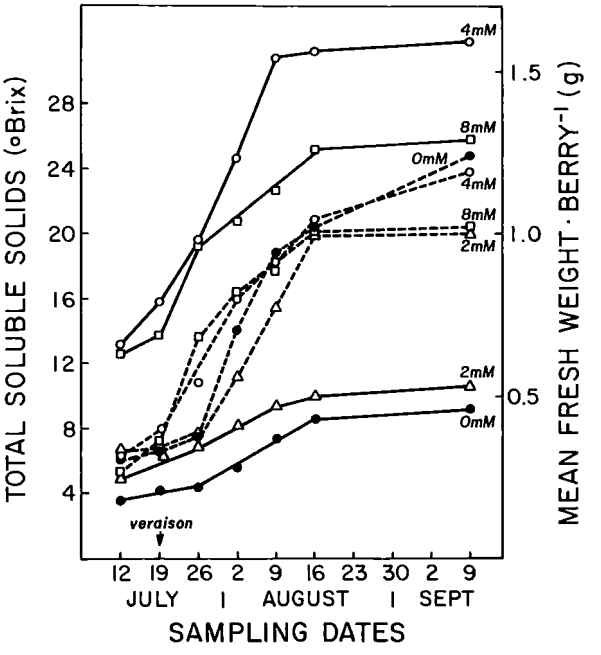

Fig. 1: Total soluble solids (dotted lines) and mean fresh weight per berry (solid lines) in *V. vinifera* L. cv. Chenin blanc as affected by the level of N fertilization.

Mostgewicht (unterbrochene Linien) und durchschnittliches Frischgewicht je Beere (durchgezogene Linien) bei *V. vinifera* L. Chenin blanc in Beziehung zur Höhe der Stickstoffdüngung.

Results

Changes in the level of total soluble solids and fresh berry weight during fruit development and ripening under four different levels of nitrogen are presented in Fig. 1. There were no consistent differences in the level of total soluble solids in berries between nitrogen treatments, however mean fresh weight per berry increased markedly in response to nitrogen, with maximum berry weight obtained at 4 mM NO_3^- (Fig. 1).

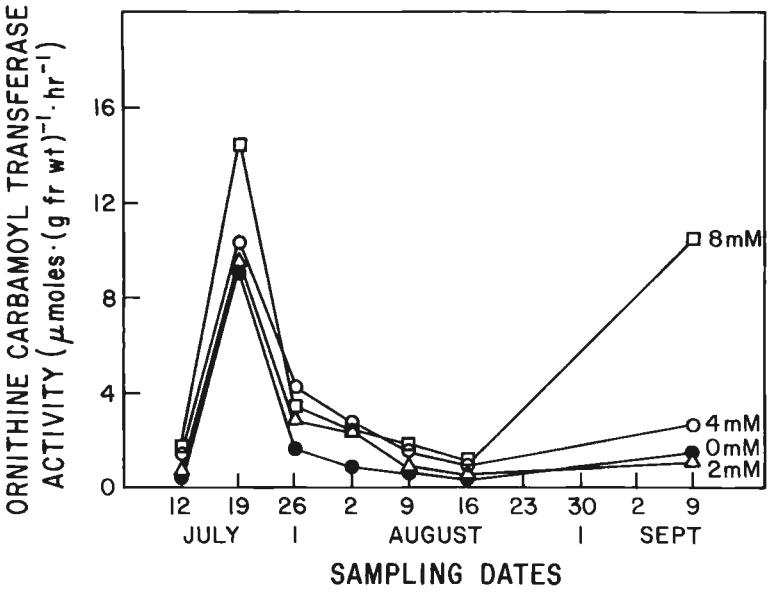

Fig. 2: Effect of N fertilization on *in vitro* ornithine transcarbamoylase activity in developing *V. vinifera* L. cv. Chenin blanc berries.

Einfluß der Stickstoffdüngung auf die Aktivität von Ornithintranscarbamylase (OTC) *in vitro* bei sich entwickelnden Beeren von *V. vinifera* L. Chenin blanc.

Changes in OTC activity in Chenin blanc berries grown under four levels of nitrogen, from July 12 to September 9, are presented in Fig. 2. At veraison, a sharp increase in OTC activity occurred in fruit grown under each of the four nitrogen regimes; thereafter, activity decreased significantly and continued to decrease for the following 4 weeks. At fruit maturity OTC activity showed a second smaller increase, the response being especially pronounced for the 8 mM NO_3^- treatment. At each sampling date enzyme activity increased with increasing rates of nitrogen.

Fig. 3 shows changes in arginase activity in berries during fruit ripening from vines grown under four different nitrogen regimes. Maximum arginase activity in berries grown with 0, 2 and 4 mM NO_3^- occurred at veraison, whereas in 8 mM NO_3^- fruit maximal enzyme activity occurred at fruit maturity (24 °Brix) with a smaller peak at veraison. Nitrogen fertilization did not basically affect the pattern of ar-

ginase activity during ripening. However, arginase activity was generally higher in 4 and 8 mM NO_3^- berries than in 0 and 2 mM NO_3^- fruit at most sampling dates (Fig. 3).

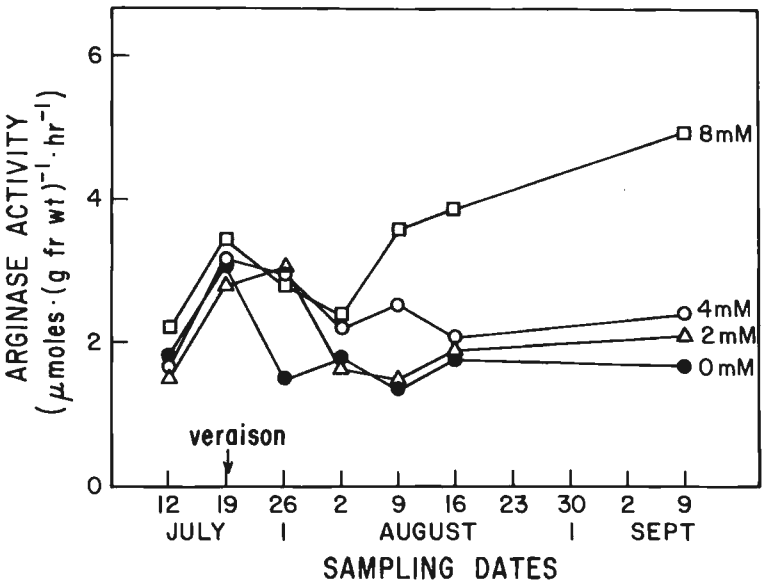

Fig. 3: Effect of N fertilization on *in vitro* arginase activity in developing *V. vinifera* L. cv. Chenin blanc berries.

Einfluß der Stickstoffdüngung auf die Arginaseaktivität *in vitro* bei sich entwickelnden Beeren von *V. vinifera* L. Chenin blanc.

Discussion

In previous papers the presence of OTC and arginase in grapevine tissues were reported along with some of their properties (8, 10). This study confirmed the earlier findings, i.e. L-arginine at concentrations greater than 30 mM caused partial inhibition of arginase; that L-ornithine at a concentration of 20 mM caused a 21.6 % inhibition of OTC and that incubation with 5 mM Mn^{++} for 1 h had no further activation effect on arginase.

The different patterns of OTC and arginase activities during the development and ripening of Chenin blanc grape berries suggest that their activities correlate with the amount of the enzyme(s) present in the tissues rather than simply reflect differences in chemical and physical nature of the berries (2). If we assume that *in vitro* OTC activity reflects the *in vivo* rate of arginine synthesis in grape berries, then this amino acid should be synthesized in greater amounts during the early stages of berry ripening (Fig. 2). However, this will only occur if ASA synthetase and ASA lyase, the enzymes which catalyze the next two reactions of the urea

cycle that lead to the synthesis of arginine, also show similar increases in their activities at veraison. Previous work revealed that these two enzymes in grapevine leaf tissue exhibited much lower activities than OTC and arginase (9). On the other hand, citrulline had no inhibitory effect on ASA synthetase activity when it was present at concentrations higher than the optimal (9).

Kliewer (3) found that arginine was depleted from roots at the time of its accumulation in fruits. However, Roubelakis-Angelakis and Kliewer (11) showed that arginine was present in relatively small amounts in xylem sap of Thompson Seedless grapevines at budbreak, whereas glutamine was present in relatively large amounts. Whether this relationship holds latter in the season is not known, nor is it known what proportion of fruit arginine is synthesized *in situ* and what proportion translocates to fruit from other parts of the vine.

In an earlier study, Kliewer and Cook (7) found that nitrate concentration in nutrient solution up to 4 mM was positively correlated with total vine growth, shoot growth, leaf area per vine, pruning weight and chlorophyll content of treated Thompson Seedless grapevines. Fresh weight per berry also increased with increasing NO_3^- in nutrient solution from 0.5 to 8 mM, whereas there was no consistent effect on the level of total soluble solids in grape berries (6, 7). In the present study, weight of Chenin blanc berries increased up to 4 mM NO_3^-, in agreement with findings obtained earlier with Thompson Seedless vines (6). Vines irrigated with 0 and 2 mM NO_3^- had considerable number of seedless berries indicating that unfavorable nutritional conditions for fertilization of ovules existed under the low nitrogen regimes. On the other hand, 8 mM NO_3^- apparently was above the optimum for maximum berry growth, since berry weight during the last 4 weeks of ripening averaged about 20 % less than in vines irrigated with 4 mM NO_3^-. Visual observations also indicated somewhat less shoot growth with 8 mM NO_3^- than with 4 mM NO_3^-.

Summary

The activities of ornithine transcarbamoylase (OTC) and arginase enzymes in Chenin blanc berries from potted vines irrigated with four different levels of nitrogen (0, 2, 4 and 8 mM NO_3^-) were determined periodically through the period of fruit ripening. OTC activity was maximal at veraison, declined rapidly thereafter with a second smaller peak of activity at fruit maturity. Arginase also generally showed maximal activity at veraison and then decreased gradually until fruit maturity, except for 8 mM NO_3^- fruit, which showed increasing arginase activities during the last 5 weeks of fruit ripening. Activities of both OTC and arginase generally increased with increasing level of nitrogen fertilization. Berry weight increased up to 4 mM NO_3^-; however, nitrogen had no consistent influence on the level of total soluble solids in fruits.

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