S3-038

Overexpression of violaxanthin de-epoxidase inhibits tobacco production

W-H Sun, AD Hieber, HY Yamamoto

Department of Molecular Biosciences and Biosystems Engineering, University of Hawaii, Honolulu, HI, 96822, USA. Fax: (808) 956-3542; email: wenhao@hawaii.edu, hieber@hawaii.edu, yamamoto@hawaii.edu

Keywords: antheraxanthin, biomass, growth, nonphotochemical quenching, xanthophyll cycle, zeaxanthin

Introduction

Violaxanthin de-epoxidase (VDE) catalyzes the conversion of violaxanthin (V) to antheraxanthin (A) and zeaxanthin (Z) in the xanthophyll cycle (Yamamoto et al. 1962). The formation of A and Z is associated with the discharge of excess photon-flux energy as heat in the light-harvesting antenna. This energy dissipation, measured as non-photochemical quenching (NPQ), protects the photosystem against the potential deleterious effects of excess light. Black et al. (1995) correlated higher productivity of rice cultivars to higher Z and A levels at midday, suggesting the possibility that VDE activity can increase photosynthesis and grain production. However, it is not yet established that increasing xanthophyll-cycle-mediated NPQ can affect plant productivity.

Genetically-engineered tobacco plants with reduced and enhanced levels of VDE have been developed in our laboratory. Previous field experiments using VDE-antisense tobacco plants demonstrated that suppressing VDE activity and, in turn, the resulting low levels of A and Z are not critical for photoprotection under field conditions (Sun et al. 2001). To determine whether increasing VDE over endogenous levels affects growth, we grew VDE-overexpressed tobacco plants in the field and examined photosynthesis and growth relative to vector-control plants.

Materials and methods

VDE-sense tobacco (*Nicotiana tabacum* cv. Xanthi) plants transformed with full-length *Arabidopsis* VDE cDNA under control of the double 35S CaMV promoter (TSS27) and vector-control plants with only the promoter (pBNG3) were used (Hieber et al. 2001). The day of sowing is defined as day zero of growth. The leaves were numbered starting from the top of the plant with leaf number 5 being the first leaf with a midrib length of about 9.5 cm (Bugos et al. 1999). The field experiment was conducted at the Waimanalo Experimental Station, College of Tropical Agriculture and Human Resources, University of Hawaii and set out in a randomized complete-block design with six replications per treatment and six plants per replicate. Methods for plant culture, pigment analysis, measurements of chlorophyll fluorescence, VDE activity and growth were as described in

Sun et al. (2001). The rate of CO_2 uptake was measured in sunlight with a Photosynthesis System CI - 301PS (CIS, Inc., Vancouver, WA).

Results and Discussion

Violaxanthin de-epoxidase activity. VDE specific activity based on total extracted thylakoid protein from leaf 18 of 71-day-old plants increased approximately13-fold in VDE-sense plants relative to vector-control plants (Figure 1A). The de-epoxidation state, (A + Z)/(V + A + Z) at midday (photon flux density (PFD) from 1600 to 1900 μmol m^{-2} s^{-1}) in leaf 18 of VDE-sense plants was 30% higher than vector-control plants (Figure 1B). In contrast, xanthophyll-cycle pool size (V + A + Z), neoxanthin, lutein, β-carotene, chlorophyll *a* and chlorophyll *b*, between control and sense plants were not significantly different (data not shown). The results show that increasing VDE above endogenous levels enhances the extent of de-epoxidation in field-grown plants as it did in growth-chamber plants (Hieber et al. 2001).

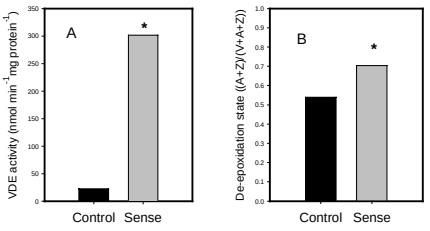

Figure 1. Violaxanthin de-epoxidase (VDE) activity on a basis of total extracted thylakoid protein (A) and de-epoxidation state (B) in leaf number 18 of vector-control (control) and VDE-sense (sense) tobacco plants at 71 days of growth. Data are means of 6 leaf samples from different plants. An asterisk indicates significance at $P \leq 0.05$ by t-test.

Fluorescence and photosynthesis. Chlorophyll-fluorescence parameters of vector-control and VDE-sense plants on attached leaves were examined at midday (PFD 1800 to 2000 μmol m^{-2} s^{-1}). The NPQ was high and similar in both types of plants, 2.3 ± 0.2 for control plants, and 2.4 ± 0.5 for VDE-sense plants. Other chlorophyll-fluorescence parameters, such as photochemical quenching (qP, 0.36 ± 0.01), maximum photochemical efficiency (0.82 ± 0.01) and midday yield (0.38 ± 0.01), recovery of photochemical efficiency after 70-min dark relaxation (91 ± 0.5%), and the photosynthetic rates (20.5 ± 0.5 μmol CO_2 m^{-2} s^{-1}) at light intensity (2130 ± 300 μmol m^{-2} s^{-1}) were similar in the vector-control and VDE-sense plants.

Fluorescence parameters were examined in leaves of outdoor-acclimated tobacco plants at PFDs' from 250 to 1600 μmol m^{-2} s^{-1} during the morning hours. As was the case at midday, no difference in NPQ, qP and yield between the sense and control plants was observed (data not shown). In addition, the kinetics of fluorescence parameters were examined at PFDs' of 360, 780 and 1700 μmol m^{-2} s^{-1} in the laboratory on leaves from dark-adapted outdoor-grown plants. The sense plants displayed faster rates of NPQ formation in the initial 1.3 min relative to the control. Moreover, this initial rapid increase of NPQ was almost identical in the VDE-sense plants at the three levels of PFD. (Figure 2A), suggesting that NPQ formation in sunlight-adapted VDE-sense plants is saturated at light intensities below 360 μmol m^{-2} s^{-1}. The final NPQ in VDE-sense plants

exposed to high and medium light were equivalent and much higher than in plants exposed to low light (Figure 2A). In vector-control plants, NPQ was initially lower than VDE-sense plants at all light levels but under high and medium light increased gradually during the course of illumination, approaching the levels in VDE-sense plants (Figure 2A). In VDE-sense plants, photochemical quenching was higher than in the control at the three levels of PFD (Figure 2B), while photochemical efficiency (yield) was close to the control (Figure 2C).

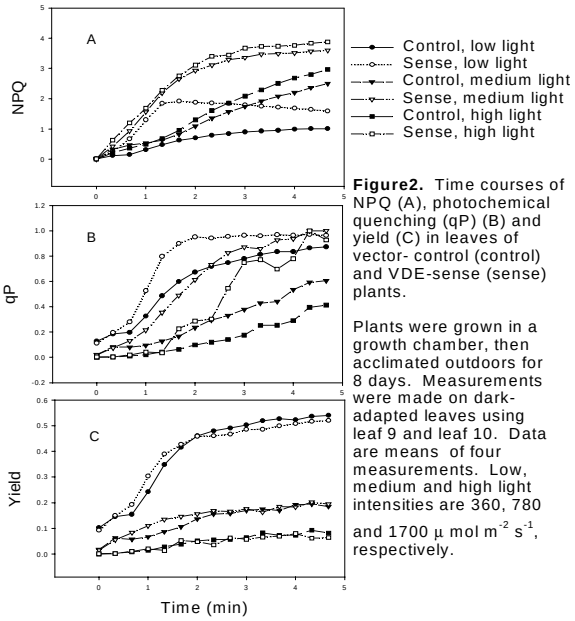

Figure2. Time courses of NPQ (A), photochemical quenching (qP) (B) and yield (C) in leaves of vector- control (control) and VDE-sense (sense) plants.

Plants were grown in a growth chamber, then acclimated outdoors for 8 days. Measurements were made on dark-adapted leaves using leaf 9 and leaf 10. Data are means of four measurements. Low, medium and high light intensities are 360, 780 and 1700 μ mol m^{-2} s^{-1}, respectively.

A typical light-response curve of NPQ is biphasic. The rapid phase is controlled by PsbS and Z and A formed from V (Li et al. 2000; Niyogi et al. 1998; Schindler and Lichtenthaler 1996); the slow phase is suggested to be from the *de novo* biosynthesis of Z (Schindler and Lichtenthaler 1996). In VDE-sense plants the rapid initial NPQ formation apparently is due to the increased rates of de-epoxidation. Lack of an increase in the final extent of NPQ in the presence of increased A+Z in the VDE-sense plants suggests a possible saturation effect. Alternatively the additional A+Z formed in VDE-sense plants may be localized where it cannot form NPQ.

Table 1. Comparison of growth parameters of vector-control and VDE-sense tobacco plants grown under field conditions. The plants were grown for 76 days (A) and 91 days (B). Each plot (replicate) contains 6 plants and six replicates per treatment. Values are means of total replicates per plant, and the standard deviation is indicated in parenthesis. An asterisk indicates significance between VDE-sense and vector-control plants within the same harvest at $P \leq 0.01$ by t-test. "NM" indicates not measured.

	Plant height (cm)	Total leaves (count)	Total leaf area (dm^2)	Dry weight (g)	
				Total lateral shoots	Total leaves
(A) 76-day-old plants					
Vector-control	120.7 (5.3)	36.5 (0.8)	170.3 (7.3)	1.6 (0.7)	60.1 (3.0)
VDE-sense	121.1 (3.0)	34.5 (0.7)*	151.7 (8.9)*	2.1 (0.6)	52.9 (3.6)*
(B) 91-day-old plants					
Vector-control	NM	33.8 (0.9)	201.2 (3.0)	32.5 (5.9)	77.9 (2.2)
VDE-sense	NM	32.1 (0.5)*	189.2 (7.8)*	33.6 (2.8)	72.2 (3.3)*

Plant growth. The first harvest was performed at day 76 when the plants were at the end of vegetative-growth stage. Total leaf number, total leaf area and total leaf dry weight per plant in VDE-sense plants were significantly lower than vector-control plants, while plant height and total lateral shoot biomass per plant were similar. These observations were confirmed by the results of the second harvest at day 91 (Table 1).

Overexpression of VDE decreased plant growth (Table 1). Although NPQ in VDE-sense plants was higher than in control plants during approximately 5-min of illumination (Figure 2B), no difference was observed in the field. This appears to rule out the possibility of the early NPQ having an effect on productivity. It is also unlikely that sustained reduction of V under high light affected growth through an effect on abscisic acid level. The level of neoxanthin in the VDE-sense plants was as high as in the vector-control and should have been sufficient to serve as a precursor for the production of abscisic acid. Time for flowering initiation was same for VDE-sense and vector-control plants (data not shown), indicating both plants developed similarly. The average leaf area in VDE-sense plants was higher than in the vector-control (data not shown). These results suggest that inhibition of leaf growth occurred early in vegetative stage and that the reduced growth may have been an effect of VDE overexpression. Further investigation is needed to determine the mechanism for the apparent relationship between VDE overexpression and reduced plant production. In conclusion, increasing VDE over endogenous level increases the rate and extent of A + Z formation. It does not increase the extent of NPQ and plant productivity, but instead, appears to inhibit tobacco production under field conditions.

Acknowledgments

Dr. RC Bugos provided valuable comments and suggestion on this report. This research was supported by USDA NRI Competitive Grant Program Agreement No. 97-35100-4851.

References

Black DCC, Tu Z-P, Counce PA, Yao P-F, and Angelov PF (1995) *Photosynthesis Research* 46: 169-175.
Bugos RC, Chang S-H and Yamamoto HY (1999) *Plant Physiology* 121:6320-6325.
Hieber AD, Bugos RC, Verhoeven AS and Yamamoto HY (2001) *Proceedings of 12th International Congress on Photosynthesis*, Brisbane, abstract S3-018.
Li X-P, Björkman O, Shih C, Grossman AR, Rosenquist M, Jansson S and Niyogi KK (2000) Nature 403: 391-395.
Niyogi KK, Grossman AR and Björkman O (1998) *Plant Cell* 10:1121-1134.
Schindler C and Lichtenthaler HK (1996) *Journal of Plant Physiology* 148: 399-412.
Sun W-H, Verhoeven AS, Bugos RC and Yamamoto HY (2001) *Photosynthesis Research* 67: 41-50.
Yamamoto HY, Nakayama TOM and Chichester CO (1962) *Archives of Biochemistry and Biophysics* 97:168-173.