Effects of temperature and CO₂ enrichment on kinetic properties of NADP⁺-malate dehydrogenase in two ecotypes of Barnyard grass (Echinochloa crus-galli (L.) Beauv.) from contrasting climates

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Summary. The apparent energy of activation (Ea), Michaelis-Menten constant (Km for oxaloacetate), Vmax/Km ratios and specific activities of NADP⁺-malate dehydrogenase (NADP⁺-MDH; EC 1.1.1.82) were analyzed in plants of Barnyard grass from Québec (QUE) and Mississippi (MISS) acclimated to two thermoperiods 28/22°C, 21/15°C, and grown under two CO₂ concentrations, 350 μl l⁻¹ and 675 μl l⁻¹. Ea values of NADP⁺-MDH extracted from QUE plants were significantly lower than those of MISS plants. Km values and Vmax/Km ratios of the enzyme from both ecotypes were similar over the range of 10–30°C but reduced Vmax/Km ratios were found for the enzyme of QUE plants at 30 and 40°C assays. MISS plants had higher enzyme activities when measured on a chlorophyll basis but this trend was reversed when activities were expressed per fresh weight leaf or per leaf surface area. Activities were significantly higher in plants of both populations acclimated to 22/28°C. CO₂ enrichment did not modify appreciably the catalytic properties of NADP⁺-MDH and did not have a compensatory effect upon catalysis or enzyme activity under cool acclimatory conditions. NADP⁺-MDH activities were always in excess of the amount required to support observed rates of CO₂ assimilation and these two parameters were significantly correlated. The enhanced photosynthetic performance of QUE plants under cold temperature conditions, as compared to that of MISS plants, cannot be attributed to kinetic differences of NADP⁺-malate dehydrogenase among these ecotypes.

Key words: Echinochloa crus-galli – Ecotypes – Temperature – CO₂ enrichment – C₄ metabolism – NADP⁺-malate dehydrogenase – Energy of activation – Km, Vmax/Km

Most C₄ species are confined to tropical or sub-tropical regions and are usually inhibited by low temperatures (Long 1983; Pearcy and Ehleringer 1984). Only a few of these species have distributions covering several climatic regions (Teeri and Stowe 1976; Caldwell et al. 1977; Teeri 1979). The C₄ weed Echinochloa crus-galli (L.) Beauv., (Barnyard grass), is such a widely distributed species with populations colonizing disturbed and agricultural habitats from tropical to cold-temperature regions (Gould et al. 1972; Maun and Barrett 1986). Previous studies of this series have compared various aspects of the C₄ metabolism of populations of Echinochloa crus-galli collected from sites with contrasting climates (e.g. Robert et al. 1983; Simon et al. 1984a, b; Potvin et al. 1986; Simon 1987; Potvin 1986, 1988a, 1988b). Reciprocal transplant experiments with plants from Québec (QUE), North Carolina (NC) and Mississippi (MISS) have shown clear ecotypic differentiation as a function of climate (Potvin 1986). Under controlled conditions, analyses of photosynthetic parameters (Potvin 1985a, b; Potvin and Strain 1985a, b; Potvin 1987, 1988b), carbon translocation (Potvin et al. 1985; Potvin 1988a), growth and resource allocation (Potvin 1985a; Potvin and Strain 1985a, b) as well as activity and kinetic properties of C₄ enzymes (Simon et al. 1984a, b; Potvin et al. 1986; Simon 1987) have demonstrated that warm-adapted MISS plants are more affected by low temperatures than cold-adapted QUE plants. A recent analysis of chilling effects on the activity of the C₄ enzymes of QUE and MISS plants, suggests that two steps of the C₄ pathway exert regulatory control on photosynthesis: Pyruvate Pi dikinase (PPDK) and NADP⁺-malate dehydrogenase (NADP⁺-MDH). The former acts through its low, rate-limiting, activity and the latter through its great cold-sensitivity. Among the four C₄ enzymes, NADP⁺-MDH is the most cold-labile and shows a highly significant reduction of activity in MISS plants compared to QUE plants following cold treatments (Potvin et al. 1986; Simon 1987).

The main objective of this particular study is to analyze possible differences in the kinetic properties of NADP⁺-MDH extracted from the two Echinochloa populations which may contribute to the enhanced photosynthetic performance of QUE plants under cold temperature conditions.

As a correlated objective, we document the effect of CO₂ enrichment on kinetic properties of NADP⁺-MDH. Recent reports indicate the existence of strong interactions between CO₂ enrichment and temperature effects on growth and photosynthesis of chilled plants (Sionit et al. 1981; Potvin and Strain 1985a, b). Previous studies of this series have shown that high levels of CO₂ may buffer Barnyard grass against the detrimental effect of cold temperatures.
in terms of plant growth, resource allocation and photosynthetic performance (Potvin 1985a, b) and C$_4$ enzyme activities (Potvin et al. 1986). CO$_2$ enrichment elicited a coordinated enhancement of thermal and kinetic properties of PEP$_c$ in Mississippi plants grown under low temperature conditions (Simon et al. 1984b). As mentioned earlier, NADP$^+$-MDH is the most cold labile of the C$_4$ enzymes and could be a limiting step of the C$_4$ pathway. Consequently, we hypothesized that the better performance of chilled Echinochloa plants under enriched CO$_2$ could, in part, be due to a CO$_2$-induced improved cold-tolerance of this enzyme.

To fulfill the two main objectives of this study, plants of Barnyard grass from Québec and Mississippi were subjected to two combinations of thermoperiods and CO$_2$ regimes and effects of thermal acclimation and CO$_2$ enrichment on NADP$^+$-MDH were investigated.

**Material and methods**

Seeds (caryopses) of *Echinochloa crus-galli* var. *crus-galli* were originally collected at Ste Anne de Bellevue, near Montréal, Québec (QUE; Lat. 45°35' N, Long. 73°50' W.) and Leland, Mississippi (MISS; Lat. 33°20' N., Long. 90°75' W.). Monthly minimum and maximum average temperatures during the growing season of the plants obtained from observations at the meteorological station are indicated in Fig. 1 (see also Simon et al. 1984a, b for further details). Seeds were germinated in a temperature controlled greenhouse of the Duke University Phytotron set at 29°C day/23°C night. After seven days, seedlings of each population under field conditions are indicated in Fig. 1 (see also Simon et al. 1984a, b for further details). Seeds were germinated in a temperature controlled greenhouse of the Duke University Phytotron set at 29°C day/23°C night. After seven days, seedlings of each population were transferred to controlled growth chambers set at two different thermoperiods 21°C/17°C and 28°C/17°C (day/night), a photoperiod of 14 h with an average photosynthetic photon flux density of 1000 μmol/m$^{-2}$s$^{-1}$ (400 W HID lights, 1/2 light pressure sodium and 1/2 multivapor) and 70% relative humidity. The two thermoperiods simulate daily mean minimum and maximum temperatures for July and August for the two sites of origin (Schwartz 1977) since it has been reported that the distribution of C$_4$ grasses is best correlated with night temperature during the growing season (Teeri and Stowe 1976). Under such conditions, plants were grown at two different CO$_2$ conditions, 350 μl l$^{-1}$ and 675 μl l$^{-1}$ (Helmers and Gilles 1979). After a period of acclimation of 5 weeks, 5 g of leaves of each subpopulation were collected at 10:00 h from the top most part of the plants which had already been illuminated for 4 h. Leaf samples were kept under constant illumination (650 μmol m$^{-2}$s$^{-1}$) during the short period of manipulations, before and during extraction procedures. Leaf segments were rapidly and thoroughly homogenized in a cold mortar with 0.5 g insoluble polyvinylpyrrolidone (PVP), 3 g sand to which were added by steps a total of 25 ml of cold extraction medium containing 50 mM HEPES-KOH, pH 7.4, 10 mM MgCl$_2$, 5 mM dithiothreitol (DTT) and 2 mM Na-EDTA (Edwards et al. 1982; Simon 1987). Triton-X (0.5%, w/v) was added after filtration through miracloth (Edwards et al. 1982). Extracts were centrifuged for 3 min at 15000 g and the supernatant was quickly filtered through a column of Sephadex G-25 equilibrated with the extraction buffer (Simon et al. 1984b). NADP$^+$-MDH preparations, diluted if necessary to give a change in absorbance per min of ca. 300 units at 30°C, were subsequently assayed spectroscopically at 340 nm according to a procedure slightly modified from Nakamoto and Edwards (1983) as described by Potvin et al. (1986) and Simon (1987).

To activate the enzyme, a 15 min incubation period under illumination preceeded assays which were initiated by the addition of oxaloacetate buffered at pH 7, which, depending on the assay, ranged from 2 to 0.0625 mM OAA. Apparent energies of activation (E$_a$) and the Michaelis-Menten constant (apparent $K_m$) were calculated as described by Simon et al. (1984b). NADP$^+$-MDH activities were expressed on a mg$^{-1}$ chlorophyll, g$^{-1}$ fresh weight leaf and dm$^{-2}$ leaf area basis as described by Simon et al. (1984b). Chlorophyll concentrations were calculated from sample extracts taken before centrifugation as recommended by Bruinsma (1963). Leaf areas were measured with a leaf area meter Li-Cor Model 3100. Assays were replicated at least two times, with readings not exceeding 10% error, and repeated on at least four independently prepared leaf extracts taken from a minimum of four plants. Photosynthesis was monitored as described in Potvin et al. (1986).

Measurements were made at 30°C in either 350 or 675 μl l$^{-1}$ CO$_2$.

Statistical analyses were performed using mixed-model ANOVAs for enzyme activities and chlorophyll concentration and MANOVA for $E_a$, $K_m$ and $V_{max}/K_m$. The main effects tested by ANOVA were population, growth temperature and CO$_2$ concentration. The only fixed effect, CO$_2$ concentration, was tested using quasi F-ratios (Winer 1971). The other enzyme properties were analyzed by MANOVA, the dependent variables being responses at each assay temperatures and the independent variables population, growth temperature and CO$_2$ concentration.

**Results**

*Apparent activation energy (E$_a$)*

$E_a$ values based on ln $Q_{10}$ estimates over a temperature range of 10–35°C are given in Fig. 2. Values for $Q_{10}$ (40/30°C) were negative for several assays, and were omitted from the figure. $E_a$ ranged from 20.3–57.9 KJoules/mole, values being lower as the assay temperature range of $Q_{10}$ increased. These differences are attributed to discontinuities in the linear relationship between activity values and assay temperatures as observed in Arrhenius plots (Simon et al. 1984b). The highest $E_a$ values obtained in this study were lower than that reported for NADP$^+$-