Thermal Dependence of Malate Synthase Activity and Its Relationship to the Thermal Dependence of Seedling Emergence

James R. Mahan†

Plant Stress and Germplasm Development Unit, Agricultural Research Service, U.S. Department of Agriculture, Route 3, Lubbock, Texas 79401

Cotton yields are often reduced by low temperatures at early planting dates. Improved seedling metabolism at low temperatures may enhance seedling performance. The glyoxylate cycle plays a role in the metabolism of stored lipids, and thus thermal limitations on the function of malate synthase (EC 4.1.3.2) may be involved in low-temperature limitations on seedlings. The thermal dependencies of the apparent K_M and maximal velocity for the malate synthases from cotton (*Gossypium hirsutum* L.) and sunflower (*Helianthus annuus* L.) were determined across a 15−45 °C thermal range and used to estimate the thermal dependence of reaction velocity. The thermal dependence of seedling emergence was monitored for both species. The thermal dependencies of predicted reaction velocity and the measured rates of seedling emergence are correlated (cotton $r^2 = 0.9$, sunflower $r^2 = 0.76$) and suggest that the thermal dependencies of enzymes predicted from basic kinetic parameters may be useful indicators of the thermal dependence of more complex whole-plant processes.

Keywords: *Cotton; malate synthase; temperature; sunflower; seedling emergence; temperature stress; glyoxylate cycle*

INTRODUCTION

On the Southern High Plains of Texas, planting of cotton into cool (<18 °C) soils (Gipson, 1986) often results in delayed seedling emergence and increased susceptibility to both disease and abiotic stress (e.g., hail, wind, and blowing soil). For farmers to have a chance at high-quality lint development later in the growing season, the establishment of vigorous, healthy seedlings is required. Temperature plays a major role in the germination and emergence of cotton seedlings. The optimum temperature for cotton germination is 30−35 °C, although normal but slower germination can occur at 18 °C. Cotton on the high plains of Texas is typically planted when the average temperature in the seed bed is 18−20 °C, clearly below what is considered to be optimal (Gipson, 1986). Early-season temperature stresses often result in reduced yield and fiber quality (Wanjura et al., 1969). Effects of low temperatures on the germination and early growth of cotton seedlings have been previously described (Meryl et al., 1986; Kerby et al., 1989; Steiner and Jacobson, 1992).

The thermal dependence of seedling emergence is a complex phenomenon involving numerous processes that are thermally dependent in and of themselves. The effect of temperature on seedling metabolism stems from both physical and metabolic thermal dependencies. Imbibition rates are thermally dependent, generally increasing with increased temperature. For example, the time required for imbibition in pea varied from 10 h at 36 °C to 16 h at 2 °C (Mayer and Poljakoff-Mayber, 1989). Soil strength, another factor that has the potential to reduce emergence rates, is not generally affected by temperature. The seed coat presents another physical impedance to seedling emergence. Because the hardness of the seed coat can affect the imbibition rate and the imbibition rate is related to temperature, higher soil temperatures would tend to diminish emergence limitations due to the seed coat. Effects of temperature on dormancy are significant in many species, although not in cotton or sunflower. Temperature-related changes in plant lipids can play a role in the thermal dependence of seedling growth. Early studies in cotton demonstrated that changes in linolenic acid in radical membranes were correlated with tolerance to chilling stress (St. John and Christiansen, 1976). The germination process, which exhibits species-specific lower and upper thermal limits, requires coordinated metabolism involving numerous enzymes and substrates, which are all thermally dependent. Temperature will affect both the capacity for and the rate of germination. Given that the seeds are planted at a temperature at which germination can occur, the rate becomes important with respect to the timely emergence of seedlings.

Simple carbohydrates in the seed are mobilized to provide energy for germination processes, whereas the more complex stored reserves (e.g., starch and lipids) begin to be mobilized as the germination process is completed. Seedlings of plants that accumulate lipids in their seeds depend on the conversion of these lipid reserves into carbohydrates for respiration and growth following germination. The glyoxylate cycle plays a central role in this metabolic conversion. The enzymes isocitrate lyase (EC 4.1.3.1) and malate synthase (EC 4.1.3.2) are unique to this metabolic pathway (Goodwin and Mercer, 1983; Trelease et al., 1987) and constitute the key linkage between β-oxidation and hexose formation (Bewley and Black, 1994). Malate synthase is present in the glyoxysomes of cotton seedlings prior to germination. Following germination, the amount of mRNA for both isocitrate lyase and malate synthase and

† E-mail jmahan@mail.csrl.ars.usda.gov.

10.1021/jf9902752
Published on Web 08/22/2000

the amount of enzyme protein increase significantly in the cotyledons. The amount and activity of these enzymes are coordinately regulated, resulting in similar temporal and spatial distributions in the plant. The gradual decline in malate synthase activity that typically begins ~3 days after imbibition indicates the transition from heterotrophic to autotrophic metabolism in the seedling. The thermal dependence of the activity of the glyoxylate cycle has been implicated in thermal effects on seedling establishment and emergence (Mohapatra et al., 1970; Scholl, 1974, 1976).

It is generally believed that improvement of low-temperature performance of cotton seedlings would result in increased profitability for producers, although attempts to identify cultivars with enhanced germination and seedling growth at low temperatures have been limited (Duesterhaus et al., 1999). In the absence of suitable cotton germplasm for low-temperature comparisons, sunflower (*Helianthus annuus* L.) was used for comparison with cotton in this study. Sunflower is an oilseed crop grown on the Southern High Plains that germinates and grows at lower temperatures than cotton (McMichael and Quisenberry, 1993; McMichael et al., 1996). Germination of sunflower is generally optimal in the range from 20 to 30 °C (Chong and Bible, 1994), significantly lower than that for cotton germination and emergence (30−35 °C). Sunflower is typically planted at soil temperatures of 10−13 °C compared with planting temperatures of 18−20 °C for cotton (Gipson, 1986). This difference in planting temperatures typically results in sunflowers being planted in mid-April as opposed to the mid-May planting dates typical for cotton.

The relationship between the thermal dependence of an enzyme and the thermal environment in which it functions has been demonstrated in various plants and animals for a variety of enzymes (Hochachka and Somero, 1984; Patterson and Graham, 1987; Simon, 1979; Simon et al., 1983). The relationship between the thermostablity of isocitrate dehydrogenases and the ecogeographic distribution of silver fir trees was investigated by Bergmann and Gregorius (1993). The maximal velocity of glutathione reductase was used by Burke and Hatfield (1987) to project differences in the ability of various species to resist thermally induced metabolic damage. Patterson and Graham (1987) used the thermal dependence of the apparent K_M to explain differences in the response of various plants to their thermal environment. Mahan et al. (1990) and Burke et al. (1988), using the thermal dependence of the apparent K_M of glutathione reductase, defined a range of optimal plant temperatures, which they termed a thermal kinetic window of optimal enzyme function (TKW). They suggested that the thermal dependence of the apparent K_M of selected enzymes could be used as an indicator of thermal stresses on enzyme-catalyzed metabolism. Mahan (1994) extended this approach by using the thermal dependencies of apparent K_M and maximal velocity to estimate enzyme reaction rates at various temperatures and substrate concentrations. In vitro measurements of the isocitrate lyase activity from cotton cotyledons were used by Mohapatra et al. (1970) to identify reductions in the activity of isocitrate lyase at low temperatures (5 °C) that were related to delayed seedling emergence. The enzyme assays in those studies were conducted at saturating substrate concentrations and thus could not detect the effects of changes in apparent K_M on reaction rate. Because substrate concentrations at the active site of many enzymes are often less than saturating, temperature-related changes in the enzyme/substrate interaction (apparent K_M) have the potential to change reaction velocity (Hochachka and Somero, 1984).

In this study, the thermal dependencies of apparent K_M and maximal velocity of malate synthase were used as previously described by Mahan (1994) to estimate the thermal dependence of reaction rates in cotton and sunflower. These estimates were compared with the thermal dependencies of seedling emergence measured at 10 °C intervals across a 15−45 °C temperature range. Comparisons of the thermal dependencies of malate synthase and seedling emergence in the two species suggest that predicted reaction rates may be useful indicators of thermal effects on seedling emergence.

MATERIALS AND METHODS

Plant Materials. Cotton (*Gossypium hirsutum* L. cv. Paymaster HS 26*) and sunflower (*Helianthus annuus* L. cv. GroAgri 422) seedlings used for enzyme analyses were grown in sand at 25 °C for 7 days prior to harvest. Seedlings for emergence studies were grown in darkness in a sand-filled thermal gradient box. A thermal gradient of 10−45 °C was generated and maintained in the sand through the use of circulating water baths. The temperature of the air was 25 °C. The plants were watered with 300 mL of deionized water on a 3 day interval.

Malate Synthase Extraction and Activity Assays. Malate synthase was extracted from cotyledons as described by Becker et al. (1978). All chemicals were purchased from Sigma Chemical Co. All steps of the extraction were performed at 4 °C. Cotyledons were homogenized in 0.05 M potassium phosphate buffer, pH 7.5 (~3 mL of buffer/g of fresh weight of cotyledons) using an Omni 1000 homogenizer. The extract was centrifuged at 14000*g* at 4 °C for 40 min, and the supernatant was assayed for malate synthase activity. The assay procedure is a modification of that described by Trelease et al. (1987). A 1 mL assay mix contained 70 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS), pH 8.2, with 0.04% Triton X-100, 0.1 mM 5,5′-dithiobis(2-nitrobenzoic acid) (prepared daily), 4 mM $MgCl_2$, 0.150 mM sodium glyoxylate (as opposed to 1.5 mM in the Trelease assay), 0.1 mM acetyl coenzyme A, and a volume of extract containing ~0.15 unit of malate synthase (1 unit of malate synthase consumes 1 μM glyoxylate per minute). Deacylase activity was accounted for with assays conducted in the absence of sodium glyoxylate. The progress of the reaction was monitored at 412 nm in a Beckman DU640 UV−vis spectrophotometer for 30 s after a 5 s equilibration period. The initial velocity of the reaction was determined with a curve fitting program. Assay temperature was controlled by a water-jacketed flow cell connected to a circulating water bath.

Determination of Kinetic Constants. The thermal dependence of maximal velocity was determined from assays at saturating substrate levels across a 15−50 °C thermal gradient. The thermal dependence of the apparent K_M for malate synthase was determined using the previously described assay with the acetyl coenzyme A concentration held constant and concentrations of glyoxylate from 4 to 1200 μM (~0.5−10 K_M). The progress of the reaction was monitored for 30 s. Apparent K_M values were determined from direct linear plots as described by Mahan et al. (1990), in which five concentrations of substrate produced eight estimates of apparent K_M at each temperature. The values of apparent K_M presented under Results represent the average of a minimum of three replicates at each temperature. The coefficient of variation for the apparent K_M values was <10% (Table 1).

Estimation of Reaction Velocity. The program for the estimation of reaction velocity was written using Stella II (High Performance Systems Inc., Hanover, NH), a model-building and simulation package. The program, which was

Table 1. Apparent K_M for Glyoxylate of Malate Synthase from Cotton and Sunflower[a]

	sunflower		cotton	
temp (°C)	apparent K_M (μM glyoxylate)	CV (%)	apparent K_M (μM glyoxylate)	CV (%)
15	12.5	9.6	86.1	4.1
20	17.4	2.8	50.4	3.7
22.5	16.7	0.5	30.6	3.3
25	16.1	2.4	13.2	3.3
27.5	21.2	1.4	8.3	2.5
30	26.2	3.1	12.8	2.3
35	54.2	3.5	17.8	2.8
40	107.1	3.8	40.3	3.5
45	124.8	4.5	91.0	4.5

[a] Apparent K_M is mean of at least three estimates. CV (coefficient of variation) is the ratio of standard error and mean.

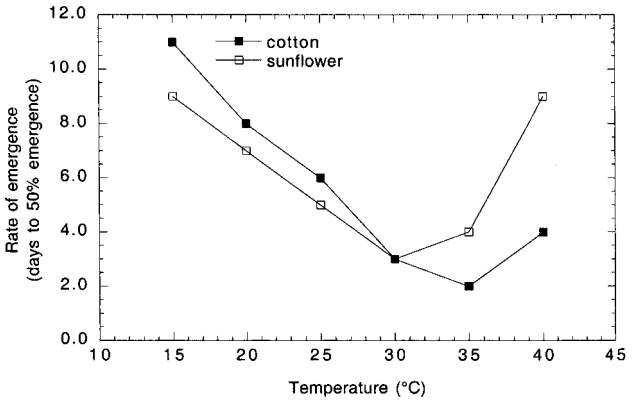

Figure 1. Effect of temperature on the rate of emergence of cotton and sunflower seedlings. Rate of emergence is expressed as the number of days required for 50% emergence. Emergence was monitored for 20 days.

previously described by Mahan (1994), utilizes the Henri–Michaelis–Menton equation to predict reaction velocity. Reaction velocity was predicted at 1 °C intervals over a thermal gradient from 15 to 45 °C. The predicted reaction velocity was calculated by the equation $v = V_{max}[S]/K_M + [S]$ from the thermal dependence of maximum velocity (V_{max}) and apparent K_M with a constant, subsaturating substrate concentration [S]. Values of apparent K_M and maximum velocity were estimated at 1 °C intervals for input into the model by piecewise linear approximation of K_M and maximum velocity values at 2.5 °C intervals. The substrate concentration for the reaction velocity predictions was set equal to the minimum K_M for each species (8.3 μM cotton and 12.5 μM for sunflower) and held constant for thermal dependence determinations.

Emergence Determination. Emergence was measured daily by counting the number of seedlings that protruded above the sand surface. The counts were continued for 20 days, and the number of seedlings emerged during that time period represented 100% emergence. The number of days to reach 50% emergence was determined from a plot of emergence as a function of days after planting.

RESULTS

The thermal dependencies of seedling emergence for cotton and sunflower are shown in Figure 1. Seedling emergence was calculated as the time required for 50% of the seedlings to emerge within 20 days. The minimum times required for 50% emergence were 2 days at 35 °C for cotton and 3 days at 30 °C for sunflower. The minimum temperature for seedling emergence within the 20-day emergence interval was 15 °C for both cotton and sunflower (emergence occurred below 15 °C but required >20 days). The upper limit for emergence was

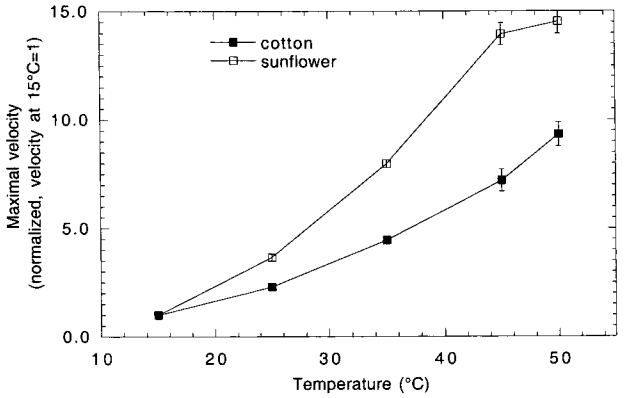

Figure 2. Thermal dependence of maximal velocity of the malate synthase from cotton and sunflower. The reaction rate at saturating substrate concentrations was measured from 15 to 50 °C.

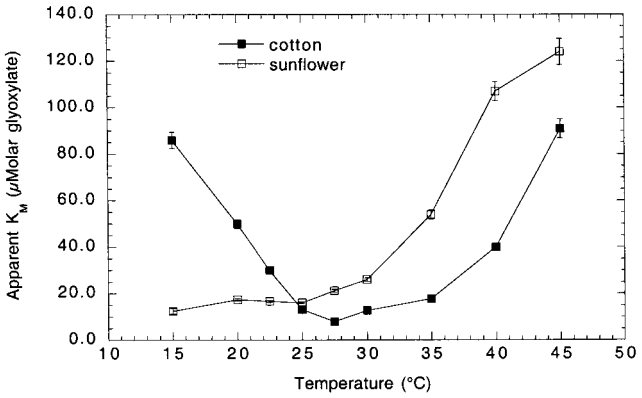

Figure 3. Thermal dependence of the apparent K_M of malate synthase for glyoxylate from cotton and sunflower. The apparent K_M was measured at temperatures between 15 and 45 °C.

40 °C for both species, above which no seedlings emerged over any time interval.

The thermal dependence of maximal velocity is shown in Figure 2. The maximal velocity of both enzymes rises with increasing temperature with an apparent "break" between 45 and 50 °C in the cotton curve, suggesting that the cotton malate synthase is more thermally labile than that from sunflower. Apparent activation energies calculated for the range from 15 to 45 °C were 53053 J mol^{-1} for cotton and 43267 J mol^{-1} for sunflower.

The apparent K_M as a function of temperature is shown in Figure 3. Cotton has a minimum value of 8.3 μM at 27.5 °C and higher values at temperatures above or below. The shape of the curve is similar to that previously reported for the glyoxylate reductase from cotton (Burke et al., 1988). The apparent K_M for sunflower malate synthase does not show the U-shaped thermal dependence of cotton; instead, the apparent K_M increases with temperature. The minimum K_M for sunflower (12.5 μM) occurred at 15 °C, the lowest temperature assayed. Similar patterns of increasing apparent K_M with increasing temperature have been reported for enzymes from other plant species (Teeri and Peet, 1978; Selinioti et al., 1986; Patterson and Graham, 1987; Mahan et al., 1990).

Predicted reaction rates, as a function of temperature, derived from a computer model of kinetic parameters are shown in Figure 4. Experimental measurements of apparent K_M and maximal velocity at each temperature were input for the model. The substrate concentration

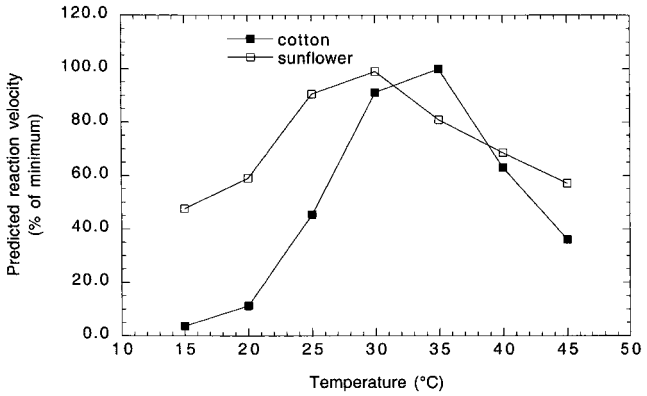

Figure 4. Thermal dependence of the reaction rate of malate synthase as predicted with a kinetic model for cotton and sunflower. The reaction rate at subsaturating substrate concentration was predicted on the basis of the thermal dependence of apparent K_M and maximal velocity.

for the simulation was set equal to the minimum observed K_M value (8.3 μM for cotton and 12.5 μM for sunflower) and held constant over the thermal range. Under the conditions of the simulation, the thermal dependence of the reaction rate for each species exhibits an apparent maximum and declines at temperatures above and below the maximum. The maximum rates for the malate synthases occurred at 35 °C for cotton and at 28 °C for sunflower.

Previous work relating the thermal dependence of enzyme kinetics to temperature stress defined ranges of optimal function for enzyme-catalyzed reactions and introduced the concept of a thermal kinetic window as an indicator of the limits of thermal stress for a plant (Burke et al., 1988; Mahan et al., 1990; Mahan, 1994). The thermal kinetic window was based upon a doubling of apparent K_M, which would result in a 33% decrease in reaction rate. In a similar manner, Mahan (1994) defined an optimal thermal range of predicted reaction velocity as the temperature range over which velocity remained within 33% of the maximum value. Optimal thermal ranges for reaction velocity of malate synthases are 26−40 °C for cotton and 21−41 °C for sunflower. Optimal thermal ranges for seedling emergence calculated in terms of a 33% increase in time required for emergence are 31−37 °C for cotton and 27−35 °C for sunflower.

A comparison of the thermal dependencies of emergence and reaction rate is shown in Figure 5. The thermal dependence of emergence was correlated with malate synthase activity with r^2 values of 0.90 for cotton and 0.76 for sunflower.

DISCUSSION

The results of this study describe differences in the thermal dependence of malate synthase from two species with differing thermal dependencies of seedling emergence. These differences are similar to those identified for various enzymes from other species in that they are (1) characteristic of the species in question and (2) to some extent indicative of the response of those species to their thermal environment (Mohapatra et al., 1970; Teeri and Peet, 1978; Selinioti et al., 1986; Burke et al., 1988; Mahan et al., 1990).

An aspect of the modeling of enzyme thermal dependence that should be discussed further is the choice of substrate concentration for the rate predictions. The

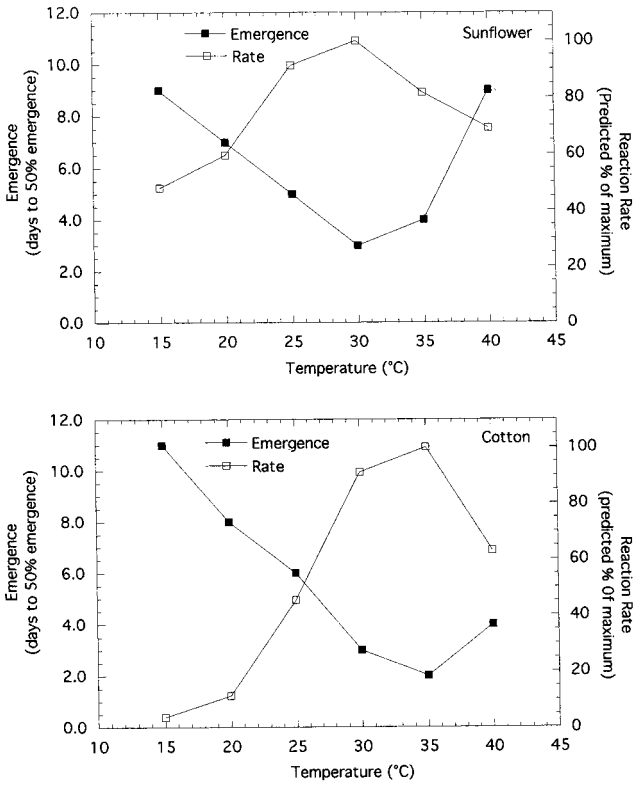

Figure 5. Seedling emergence and predicted reaction rate of malate synthase as a function of temperature for cotton and sunflower across a 15−45 °C thermal gradient.

dependence of rate on substrate concentration in the rate model is such that, at substrate concentrations which are much larger than the K_M, the thermal dependence of the rate approaches that of the maximal velocity. As substrate concentrations approach or fall below the value of the apparent K_M, the rate function is increasingly influenced by the apparent K_M. In the simulations, the substrate concentration was set equal to the minimum apparent K_M observed so that the thermal dependence of the apparent K_M influenced the reaction velocity. This decision was based upon the relationships between apparent K_M and substrate concentration described by Hochachka and Somero (1984), who suggested that substrate values in vivo tend to be similar to the K_M value under nonstressed conditions. Although measured values of substrate concentration might constitute the ideal input into the model, the correlation between the modeled and measured thermal dependencies in this study suggests that the substrate concentration assumptions used are substantially correct.

In addition to the thermal dependence of the apparent K_M and maximal velocity, which were included in this analysis, there are other cellular factors that can alter the enzyme reaction rate that also vary with temperature. Perhaps most pertinent are cellular pH and osmotic composition. Somero (1986) discussed these factors in detail and underscored their importance in the proper function of enzymes in varied environments. It is probable that variation in both pH and the concentration and composition of what he termed the "intracellular milieu" accompanied changes in environmental temperature in this study. That the thermal dependence of seedling emergence agreed with the predicted rate variation in both species suggests that,

even in the presence of mitigating factors, the thermal dependence of malate synthase has the potential to limit metabolism at some temperatures.

The ultimate usefulness of any modeling approach is limited by the extent to which the predictions agree with the behavior of the plant under realistic conditions, so the thermal dependence of seedling emergence was also determined. The correlation between the thermal dependencies of seedling emergence and malate synthase activity suggests that thermal limitations on the enzyme activity may play a role in the effects of temperature on seedling performance. The correlations between the thermal dependencies of malate synthase and seedling emergence do not establish the existence of a cause/effect relationship. However, they do agree with earlier reports of a relationship between glyoxylate cycle activity and the thermal environment of cotton seedlings which suggested that thermal limitations on glyoxylate cycle metabolism were involved, to some degree, in temperature-related delays in seedling emergence (Mohapatra et al., 1970; Scholl, 1974, 1976).

The different thermal responses of the malate synthases from the two species demonstrate the existence of genetic variability that may be useful in the application of genetic engineering approaches to the development of plants with enhanced low-temperature performance. The observed differences in the thermal dependence of the malate synthases between the species in this study and the reported successes in the transgenic expression of malate synthase in several plant species (Graham et al., 1990; Comai et al., 1992) suggest that there is potential for improvement of the thermal dependence of glyoxylate cycle activity through the expression of enzymes that are tailored to the thermal environment of early planting dates. Oliver et al. (1995) demonstrated that the thermal dependence of the pool of a single enzyme could be broadened by the interspecific transfer of genes. In their study, when a gene for hydroxypyruvate reductase from cucumber was expressed in tobacco, the thermal characteristics of the enzyme from the transgenic plants represented an equal mix of the two forms present. This established the feasibility of tailoring the thermal dependence of an enzyme to better fit the thermal environment of a plant.

Definition of the thermal environment is a necessary step in the determination of the thermal characteristics needed to match an enzyme to a specific thermal environment. Because planting occurs within a limited time frame and soil temperature is easily monitored, the thermal environment of seedlings in the field is relatively straightforward to document. Soil temperatures at 10 cm (seed depth) were monitored for the interval April 21−May 21, 1994, in Lubbock, TX (data not shown). Comparison of soil temperature with thermal ranges of 25−35 °C for sunflower (30 ± 5 °C) and 30−40 °C for cotton (35 ± 5 °C) indicated 18.6 and 2.4% of time within optimum for sunflower and cotton, respectively. These results indicate the potential magnitude of changes in thermal stress that could result from changes in thermal optima for seedling metabolism.

In summary, it has been demonstrated that the thermal dependence of the reaction rate of seedling malate synthase as predicted from kinetic parameters is correlated to that of seedling emergence. This apparent linkage suggests possible approaches for the reduction of thermal stress on cotton seedlings.

ABBREVIATIONS USED

K_M, Michaelis constant; [S], substrate concentration; V_{max}, maximal velocity; MOPS, 3-(*N*-morpholino)propanesulfonic acid.

LITERATURE CITED

Becker, W.; Leaver, C.; Weir, E.; Riezman, H. Regulation of glyoxysomal enzymes during germination of cucumber. *Plant Physiol.* **1978**, *62*, 542−549.

Bergmann, F.; Gregorius, H. R. Ecogeographic distribution and thermostability of isocitrate dehydrogenase (IDH) alloenzymes in European silver fir (*Abies alba*). *Biochem. Syst. Ecol.* **1993**, *21*, 597−605.

Bewley, J. D.; Black, M. *Seeds. Physiology of Development and Germination*; Plenum Press: New York, 1994.

Burke, J. J.; Hatfield, J. L. Plant morphological and biochemical responses to field water deficits. III. Effect of foliage temperature on the potential activity of glutathione reductase. *Plant Physiol.* **1987**, *85*, 100−103.

Burke, J. J.; Mahan, J. R.; Hatfield, J. L. Crop-specific thermal kinetic windows in relation to wheat and cotton biomass production. *Agron. J.* **1988**, *80*, 553−556.

Chong, C.; Bible, B. B. Germination and emergence. In *Handbook of Plant and Crop Physiology*; Pessarakli, M., Ed.; Dekker: New York, 1994; pp 85−146.

Comai, L.; Matsudaira, K. L.; Heupel, R. C.; Dietrich, R. A.; Harada, J. J. Expression of a *Brassica napus* malate synthase gene in transgenic tomato plants during the transition from late embryogeny to germination. *Plant Physiol.* **1992**, *98*, 53−61.

Duesterhaus, B.; Hopper, N.; Gannaway, J.; Jividen, G. Development of a laboratory screening test for the evaluation of cold tolerance in cottonseed germination. *Proceedings of the Beltwide Cotton Conference*; National Cotton Council: Memphis, TN, 1999; Vol. 1, pp 621−623.

Gipson, J. Temperature, growth, development, and fiber properties. In *Cotton Physiology*; Mauney, J. R., Sewart, J. McD., Eds.; The Cotton Foundation: Memphis, TN, 1986; pp 47−56.

Goodwin, T. W.; Mercer, E. I. *Introduction to Plant Biochemistry*, 2nd ed.; Pergamon Press: New York, 1983; p 306.

Graham, I. A.; Smith, L. M.; Leaver, C. J.; Smith, S. M. Developmental regulation of expression of the malate synthase gene in transgenic plants. *Plant Mol. Biol.* **1990**, *15*, 539−549.

Hochachka, P. W.; Somero, G. W. *Biochemical Adaptation*; Princeton University Press: Princeton, NJ, 1984; pp 389−393.

Kerby, T. A.; Keely, M.; Johnson, S. Weather and seed quality variables to predict cotton seedling emergence. *Agron. J.* **1989**, *81*, 415−419.

Mahan, J. Thermal dependence of glutathione reductase; thermal limitations on antioxidant protection in plants. *Crop Sci.* **1994**, *34*, 1550−1556.

Mahan, J. R.; Burke, J.; Orzech, K. The thermal dependence of the apparent KM of glutathione reductases from three plant species. *Plant Physiol.* **1990**, *93*, 822−824.

Mayer, A. M.; Poljakoff-Mayber, A. *The Germination of Seeds*; Pergamon Press: New York, 1989; pp 45−46.

McMichael, B. L.; Quisenberry, J. E. The impact of the soil environment on the growth of root systems. *Environ. Exp. Bot.* **1993**, *33*, 53−61.

McMichael, B. L.; Quisenberry, J. E.; Burke, J. J. Temperature effects on root growth. In *Plant Roots: The Hidden Half*; Waisel, Y., Eshel, A., Kafkafi, U., Eds.; Dekker: New York, 1996; pp 383−396.

Meryl, N.; Rowland, C.; Rowland, R. A. Germination and stand establishment. In *Cotton Physiology*; Mauney, J. R., Sewart, J. McD., Eds.; The Cotton Foundation: Memphis, TN, 1986; pp 535−541.

Mohapatra, S. S.; Smith, E. W.; Fites, R. C.; Noggle, G. R. Chilling temperature depression of isocitratase activity from

cotyledons of germinating cotton. *Biochem. Biophys. Res. Commun.* **1970**, *40*, 1253−1258.
Oliver, M.; Ferguson, D. L.; Burke, J. J. Interspecific gene transfer implications for broadening temperature characteristics of plant metabolic processes. *Plant Physiol.* **1995**, *107*, 429−434.
Patterson, B. D.; Graham, D. Temperature and metabolism. In *The Biochemistry of Plants; A Comprehensive Treatise*; Stumpf, P. K., Conn, E. E., Eds.; Academic Press: New York, 1987; Vol. 12, pp 162−165.
Scholl, R. L. Inheritance of isocitratase activity and its relationship to seedling vigor in a cross of upland cotton. *Crop Sci.* **1974**, *14*, 296−300.
Scholl, R. L. Variability within *Gossypium hirsutum* L. for seedling isocitratase activity. *Crop Sci.* **1976**, *16*, 701−703.
Selinioti, E.; Manetas, Y.; Gavalas, N. A. Cooperative effects of light and temperature on the activity of phosphoenolpyruvate carboxylase from *Amaranthus paniculatus* L. *Plant Physiol.* **1986**, *82*, 518−522.
Simon, J. P. Adaptation and acclimation of higher plants at the enzyme level: latitudinal variations of thermal properties of NAD malate dehydrogenase in *Lathyrus japonicus* Willd. *Oecologia* **1979**, *39*, 273−287.
Simon, J. P.; Potvin, C.; Blanchard, M. Thermal adaptation and acclimation of higher plants at the enzyme level: kinetic properties of NAD malate dehydrogenase and glutamate oxaloacetate transaminase in two genotypes of *Arabadopsis thaliana*. *Oecologia* **1983**, *60*, 143−148.
Somero, G. N. Protons, osmolytes, and fitness of internal milieu for protein function. *Am. J. Physiol.* **1986**, *251*, 197−213.
Steiner, J.; Jacobson, T. A. Time of planting and diurnal soil temperature effects on cotton seedling field emergence and rate of development. *Crop Sci.* **1992**, *32*, 238−234.
St. John, J.; Christiansen, M. N. Inhibition of linolenic acid synthesis and modification of chilling resistance in cotton seedlings. *Plant Physiol.* **1976**, *57*, 257−259.
Teeri, J. A.; Peet, M. M. Adaptation of malate dehydrogenase to environmental temperature variability in two populations of *Potentilla glandulosa* L. *Oecologia* **1978**, *34*, 133−141.
Trelease, R.; Hermeranth, C.; Turley, R.; Kunce, C. Cotton seed malate synthase. *Plant Physiol.* **1987**, *84*, 1343−1349.
Wanjura, D. F.; Hudspeth, E. B.; Bilbro, J. D. Emergence time, seed quality, and planting depth effects on yield and survival of cotton (*Gossypium hirsutum* L.). *Agron. J.* **1969**, *61*, 63−65.

Received for review March 17, 1999. Accepted July 15, 2000. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

JF9902752