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Interspecific variation for thermal dependence of glutathione reductase in sainfoin*

S.P. Kidambi[1], **J.R. Mahan**[2] **and A.G. Matches**[1]

[1] Department of Agronomy, Horticulture, and Entomology, Texas Tech University, Lubbock, TX 79409-4169, USA
[2] USDA-ARS Cropping Systems Research Laboratory, Route 3, Box 215, Lubbock, TX 79401-9757, USA

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Summary. Understanding the biochemical and physiological consequences of species variation would expedite improvement in agronomically useful genotypes of sainfoin (*Onobrychis* spp.). Information on variation among sainfoin species is lacking on thermal dependence of glutathione reductase (E.C. 1.6.4.2.), which plays an important role in the protection of plants from both high and low temperature stresses by preventing harmful oxidation of enzymes and membranes. Our objective was to investigate the interspecific variation for thermal dependency of glutathione reductase in sainfoin. Large variation among species was found for: (i) the minimum apparent Km (0.4–2.5 μM NADPH), (ii) the temperature at which the minimum apparent Km was observed (15°–35 °C), and (iii) the thermal kinetic windows (2°–30 °C width) over a 15°–45 °C temperature gradient. In general, tetraploid species had narrower ($\leq$17 °C) thermal kinetic windows than did diploid species ($\sim$30 °C), with one exception among the diploids. Within the tetraploid species, the cultivars of *O. viciifolia* had a broader thermal kinetic window ($\geq$7 °C) than the plant introduction (PI 212241, $\geq$2 °C) itself.

Key words: Apparent Km – Glutathione reductase – *Onobrychis* spp. – Sainfoin – Thermal kinetic window

Introduction

Sainfoin (*Onobrychis viciifolia* Scop.) is a forage legume grown primarily as animal feed. T. P. Bolger (unpublished data) reported that 'Renumex' sainfoin had a higher rate of biomass accumulation from March through May than from July through September. This difference in biomass accumulation was due to higher temperature from July through September. Temperature is one of the major environmental constraints dictating the distribution of both wild and cultivated species of sainfoin. Improvement in the ability of this crop to grow at high temperatures will enhance the feed-flow to cattle during the warm months. Thus, genetic information on thermal respones in related strains would be useful in breeding programs.

An understanding of the biochemical and physiological consequences of thermal variation among species would expedite the improvement of agronomically useful genotypes. Influence of ploidy on physiology (Pfeiffer et al. 1980) and biochemistry (Meyer et al. 1982) were studied in alfalfa (*Medicago* spp.). Several studies have shown that agronomic performance of diploid populations is inferior to genetically comparable tetraploids. The forage yield of tetraploid alfalfa is nearly twice that of diploids (Bingham and McCoy 1979). Higher ploidy may also result in more succulent forage that is higher in soluble dry matter and lower in structural constituents.

Nuclear polyploidization is an important component in the evolution of many plant species. Cultivated sainfoin is a tetraploid (2n = 4x = 28) behaving cytogenetically and genetically as an autotetraploid (Fyfe 1946), with wild diploid (2n = 2x = 14) relatives.

Glutathione reductase (GR, E.C. 1.6.4.2) plays an important role in the protection of plants from both high and low temperature stresses by preventing the oxidation of enzymes and membranes (De Kok and Oosterhuis 1983; Halliwell and Foyer 1978). Although GR has been purified and characterized from a variety of plant species (Halliwell and Foyer 1978; Kalt-Torres et al. 1984; Con-

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nell and Mullett 1986; Mahan and Burke 1987), little is known about the influence of ploidy level on thermal dependency of GR. The purification of GR from sainfoin and genetic variation for the thermal dependence were reported earlier (Kidambi et al. 1990).

Plants experience a range of temperatures both diurnally and seasonally. Hazel and Prosser (1974) and Somero (1975) demonstrated that the ability of an organism to adjust its metabolic rate to changes in temperature is strongly correlated with the degree of temperature dependence of enzyme-catalyzed reactions.

The effect of temperature on enzymes and its effect on the interaction between an organism and its environment has been extensively investigated in a variety of plant and animal systems. Though apparent Km (the concentration of substrate where the rate of reaction is half maximum) generally depends on temperature (Dixon et al. 1979), the possibility of thermal sensitivity of enzymes resulting from changes in apparent Km with temperature was ignored (Graham and Paterson 1982). Mahan et al. (1987) and Burke et al. (1988) have investigated differences among crops in the thermal dependence of the apparent Km of two enzymes. They have shown that the function of GR from corn, cotton, cucumber, wheat, and spinach species with different responses to thermal stress is limited to species-specific temperature ranges. However, such information is not available for sainfoin species. Therefore, our objective was to investigate the interspecies variation for thermal dependency of GR in sainfoin.

Materials and methods

Glutathione reductase was purified during April 1989, from about 50 g of leaf tissue from three diploid introductions [*O. grandis* (PI 297923), *O. petraea* (PI 312946 and PI 316295)] and four tetraploid introductions [*O. bieberstenii* (PI 284124), *O. tanaitica* (PI 312950), *O. transcaucasica* (PI 372827), and *O. viciifolia* (PI 212241)], and three tetraploid cultivars of *O. viciifolia* ('Renumex', 'Remont', and 'Eski'), according to the procedures described by Mahan and Burke (1987). All genotypes were field grown and were in an early vegetative stage of growth at the time of sampling.

The activity of the enzyme was monitored according to the procedure described by Kidambi et al. (1990). Briefly, the effect of temperature on the reaction was monitored using a Gilford response spectrophotometer (Gilford Instrument Labs, Oberlin/OH) over the temperature range of 15° to 45 °C using ten concentrations of NADPH, ranging from 2 to 200 μM NADPH. The selected temperatures represent the upper range to which plants are exposed in the field during the season. The lower temperature (15 °C) was used because of limitations in instrumentation. The reaction began with the addition of 0.02 units (one unit is defined as the amount of enzyme that catalyzes the reduction of 1 μM of GSSG) of purified GR. Using the first ten observations on the reaction curve, 45 values of initial velocity were calculated (Cornish-Bowden 1979). The mean value of these 45 estimates of initial velocity was used in the calculation of apparent Km by direct linear plots of substrate concentrations and initial rates, as described by Cornish-Bowden (1979). Subsequently, in this paper Km refers to the apparent Km values as calculated above.

Electrophoresis was carried out in the absence of sodium dodecylsulfate, according to the procedure described by Mahan and Burke (1987). The activity of the enzyme in both the ploidy levels and one cultivar was determined according to the procedure described by Kalt-Torres et al. (1984).

Results and discussion

Glutathione reductase from different sainfoin species was of high purity (Kidambi et al. 1990), and only one band appeared on the activity gel corresponding to a molecular weight of approximately 140-K. Hence, the enzyme is not isolated in any isozymic forms. However, isoelectric focussing would be necessary to conclude that the single band found under non-denaturing conditions was not comprised of several polypeptides. Guern and Herve (1980) reported that there were no significant differences in protein content among di-, tetra-, and hexaploid populations of *Hippocrepis comosa* L., and that the amount of DNA was not proportional to the number of genomes. They concluded that there were no gross differences in the amount or properties of aspartate-transcarbamylase with changing ploidy number. Habben and Volenec (1989) reported that ploidy had only a minor influence on amylase activity in alfalfa taproots, with much larger differences evident among populations within a ploidy level. The Km of GR in some sainfoin species initially decreased and then increased (in all species) with increasing temperature (Figs. 1 and 2). Similar behavior of the Km with respect to temperature has been reported for cucumber, spinach, wheat, cotton, and corn (Mahan et al. 1987; Burke et al. 1988).

Among the diploids, the lowest Km ranged from 1.6 to 2.0 μM NADPH, with PIs 312946 and 316295 exhibiting their lowest Kms at 15 °C, while PI 297923 had its lowest Km at 35 °C (Fig. 1).

Among the tetraploids, the lowest Km is 0.4 μM NADPH (Fig. 2). Among the four tetraploid introductions, the temperature at which the lowest Km was observed ranged from 15° to 35 °C. This suggests that the Km of GR for NADPH varies with temperature among the genotypes. However, *O. viciifolia* and the cultivars derived from this species had their minimum Kms at much lower temperatures (Fig. 3). The lowest Km observed among the tetraploids (1.3 μM NADPH) is lower than two of the diploids. This suggests that the observed lowest Km of GR for NADPH is higher in tetraploids than in diploids.

From morphological observations (leaf shape and size, and inflorescence type), *O. grandis*, *O. bieberstenii*, and *O. transcaucasica* resemble each other. Because polyploidization at the nuclear level has been suggested as an important factor in evolution of flowering plants, and

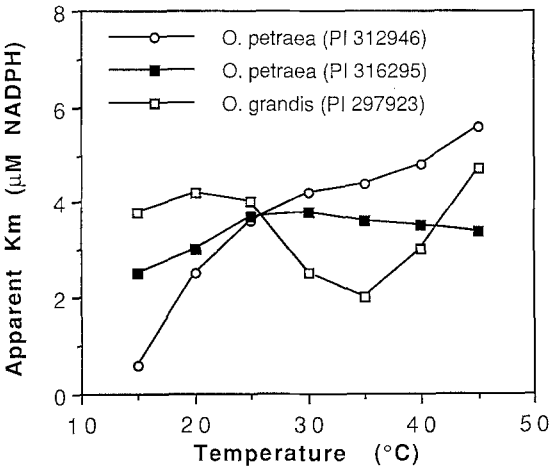

Fig. 1. The temperature dependence of the Km for NADPH of glutathione reductase from diploid sainfoin

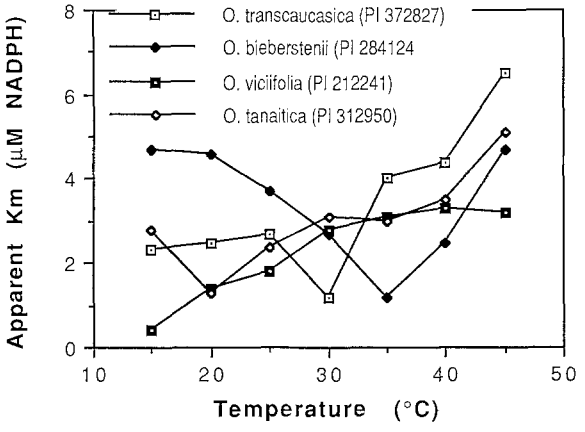

Fig. 2. The temperature dependence of the Km for NADPH of glutathione reductase tetraploid sainfoin

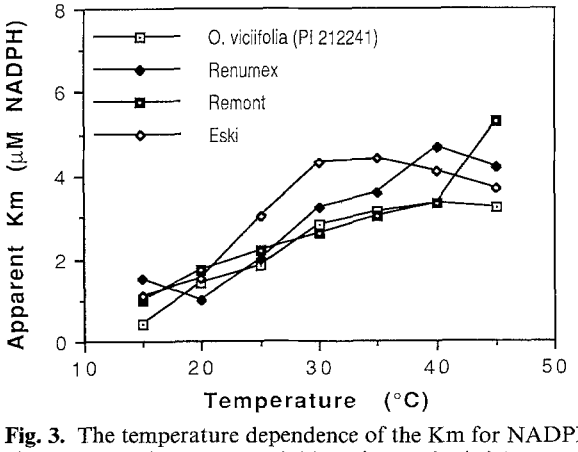

Fig. 3. The temperature dependence of the Km for NADPH of glutathione reductase tetraploid cultivars of sainfoin

because of the temperature at which the lowest Km was observed for these three species, it can be speculated that the diploid species (i.e., *O. grandis*) may have contributed its genome to the other two tetraploid species. However, an authentic confirmation is possible only with cytogenetical studies among the interspecific hybrids of these three species.

Patterson and Graham (1987) suggested that a low and constant Km may be evolutionarily conserved within the temperature range that is optimal for growth. If the minimum Km is a good indicator of the efficiency of enzymes (Teeri 1980), then GR from these species differs in the temperature optimum for their performance. In the present study we found differential sensitivity in the response of Km to temperature in both diploid and tetraploid sainfoin species.

Mahan et al. (1987) have defined the temperature range over which the Km is within 200% of the minimum observed Km as the 'thermal kinetic window' (TKW). Using this concept, Mahan et al. (1987) and Burke et al. (1988) suggested that TKWs are species specific. The TKWs for GR from diploid and tetraploid sainfoin are shown in Table 1. The diploid species exhibited a TKW of ca. 30 °C, except for one species, while all the tetraploid species had a window of $\leq$17 °C. This suggests that the increased genome size may be negatively associated with the width of the window. *If* GR functions better within TKW and *if* TKW is related to plant growth behavior (Burke et al. 1988), then the diploid species would perform better under a broader range of temperatures than tetraploid species. Also, the wider TKW for the cultivars of *O. viciifolia* as compared to the introduction may be a result of selection for improved yield. Habben and Volenec (1989) reported an increased α-amylase activity in an agronomically superior cultivar ('Hi-Phy') of alfalfa than in genetically comparable diploid or tetraploid germ plasms. It is apparent that *O. grandis*, *O. petraea*, and *O. transcaucasica* have the broadest TKWs and, therefore, would be expected to perform better than other genotypes within a variable environment. This insensitivity to temperature from the two diploids (*O. grandis* and *O. petraea*) may be transferred to the cultivated tetraploids, according to the techniques used in transferring desirable traits from wild diploid (2n = 2x = 14) *Dactylis glomerata* ssp. to cultivated tetraploid (2n = 4x = 28) orchard grass. Such an introgression of exotic germ plasm in sainfoin breeding programs should contribute useful genetic diversity to improve yield.

Fyfe (1946) suggested that the *O. viciifolia* behaved as an autotetraploid cytogenetically. If all the tetraploid species were derived from the same diploid, then there would be no variation for enzyme activity due to the genome size alone, as shown in alfalfa (Bingham and McCoy 1979). However, the variation for the TKW-re-

Table 1. Temperature at which the minimum apparent Km was observed, lower and upper limits of thermal kinetic window (TKW, range of temperature within 200% of the minimum), and the width of the windows for the glutathione reductases from diploid (2n = 2x = 14) and tetraploid (2n = 4x = 28) species of sainfoin, with countries of origin in parenthesis

Genotype	Temperature of minimum observed Km	TKW limits		Width of the window
		Lower	Upper	
	(°C)			
Diploids:				
O. grandis (PI 297923) (Australia)	35	15	43	28
O. petraea (PI 312946) (USSR)	15	≤15	23	≥8
O. petraea (PI 316295) (FRG)	15	≤15	45	≥30
Tetraploids:				
O. bieberstenii (PI 284124) (Hungary)	35	31	40	9
O. tanaitica (PI 312950) (USSR)	20	15	27	12
O. transcaucasica (PI 372827) (Czechoslovakia)	30	15	32	17
O. viciifolia (PI 212241) (Washington, USA)	15	≤15	17	≥2
Eski	15	≤15	22	≥7
Remont	15	≤15	23	≥8
Renumex	15	≤15	25	≥10

lated traits observed among the sainfoin species could be explained by the fact that the diploid wild progenitors of the tetraploid species may be different genomes. Unfortunately, there is no sufficient cytogenetic data available to prove either way.

Since our main objective was to investigate the variation among the sainfoin species for the thermal dependency of GR, we did not use any genetically comparable populations at the two ploidy levels as was done in alfalfa (Pfeiffer et al. 1980; Meyers et al. 1982; Habben and Volenec 1989). Therefore, the results from this research are to be extrapolated with caution. This study revealed large differences among diploid and tetraploid sainfoin genotypes for the minimum observed Km, the temperature at which the observed Km is minimum, and the TKWs for GR. These differences may be the result of phenotypic adaptation or genetic differences between the genotypes. Burke et al. (1988) reported a linear relationship between the total amount of time that foliage temperatures were within the TKW and plant biomass production. Under irrigated conditions, Upchurch and Mahan (1988) presented evidence that cotton plants maintain their leaf temperatures within a few degrees where the lowest Km (26°–28 °C) for glyoxylate reductase (Burke et al. 1988) was observed. Therefore, our future studies will focus on relating thermal dependence of the Km of GR and plant growth rates and forage quality of these species throughout the growing season.

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