

RELATIONSHIP BETWEEN PLOIDAL LEVEL AND CAM IN FIVE SPECIES OF *TALINUM* (PORTULACACEAE)¹

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Plants ($N = 102$) from 23 populations of *Talinum*, representing five morphologically similar species, were examined in a test of the hypothesis that ploidal level may be correlated with levels of Crassulacean acid metabolism. When grouped by species and ploidal level, two groups of tetraploid plants, *T. calcaricum* and tetraploid individuals of *T. calycinum*, had significantly greater malic acid accumulations than any other group, including diploid individuals of *T. calycinum*. Another tetraploid, *T. teretifolium*, did not show greater malic acid fluctuations than the diploids, including two putative ancestors (*T. mengesii* and *T. parviflorum*). Similarly, malic acid fluctuations in tetraploid individuals of *T. parviflorum* were not different from diploid individuals of the same species. Nonetheless, when all plants were compared, nocturnal accumulations of malic acid in tetraploid plants were significantly greater than those of diploid plants.

Polyploidy has been a major process influencing the evolution of higher plants (Stebbins, 1971). Although the occurrence of polyploidy has been documented in a wide variety of taxa, the physiological and ecological consequences of chromosomal multiplication have received little attention (Lewis, 1980; Levin, 1983; Lumaret, 1988). Increases in ploidal level have been correlated with increased or decreased drought tolerance, growth rate, and photosynthetic rate, depending on the species (Tal, 1980; Levin, 1983; Warner and Edwards, 1989). The consequences of polyploidy may also vary among different populations within the same species, as demonstrated by the differential effects of artificially induced autopolyploidy on photosynthesis in different cultivars of *Phlox drummondii* (Bazzaz et al., 1983). In spite of the apparent variability in the effects of polyploidy in different groups of plants, one consistent consequence of polyploidy is an increase in cell size (Stebbins, 1971).

Although several studies have examined the consequences of artificially derived and naturally occurring polyploidy on the physiology of related groups of plants, very few have focused on plants that utilize the Crassulacean acid metabolism (CAM) pathway of photosynthesis (Levin, 1983). In one such study, involving naturally occurring diploid, tetraploid, and hexaploid individuals of *Sedum pulchellum*, Smith (1943) found that as ploidal level increased, so did cell size, leaf thickness, and drought tolerance, while growth rates decreased. *Sedum pulchellum* performs the CAM-cycling mode of photosynthesis (Martin, Higley, and Wang, 1988b), in which atmospheric carbon uptake occurs by the C_3 pathway, concomitant with CAM acid fluctuations that result from nighttime

refixation of respiratory CO_2 (Ting, 1985; Martin, Higley, and Wang, 1988a).

Increased ploidal levels, accompanied by greater quantities of DNA in photosynthetic cells, may result in higher photosynthetic rates via increased activities of photosynthetic enzymes. On a per cell basis, for example, Warner and Edwards (1989) found increased amounts of DNA and activities of photosynthetic enzymes accompanying increases in photosynthetic rates as ploidal level increased in a naturally occurring polyploid series of the C_4 shrub *Atriplex confertifolia*.

Unlike C_3 and C_4 plants, however, photosynthetic rates in CAM plants may not necessarily increase solely as a result of increased activities of photosynthetic enzymes because of restrictions imposed by the vacuolar volume for malic acid storage during CAM (Winter, 1985). The limited space available for overnight storage of malic acid most likely limits rates of carbon uptake, and explains, at least in part, the relatively slow growth rates of CAM plants (Kluge and Ting, 1978; Winter, 1985; but see Lüttge, 1987). The relationship of tissue succulence, as a predictor of vacuolar size, to CAM was demonstrated in several species of *Sedum*, in which the degree of CAM photosynthesis, as estimated by stable carbon isotope ratios of leaf tissues, increased with leaf thickness (Teeri, Tonsor, and Turner, 1981). Thus, polyploidy in CAM species may increase photosynthetic rates as a result of larger vacuoles in photosynthetic tissue, regardless of its effect on activities of photosynthetic enzymes. Such increases in photosynthesis at a cellular level, however, may not translate into higher photosynthetic rates on a whole plant basis if there are fewer cells formed during leaf development in polyploid species (Stebbins, 1971; Warner and Edwards, 1989).

In this study, the degree of CAM was examined in naturally occurring diploid and tetraploid individuals of five closely related species of *Talinum*. All species are small, succulent plants that occur in shallow soil patches on rock outcrops throughout the eastern and midwestern United States, and all perform CAM-cycling (Harris and Martin, 1991). Although substantial physiological differences separate plants with CAM-cycling from CAM plants (Ting, 1985; Martin, Higley, and Wang, 1988a), the term

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“CAM” is used in this study solely to indicate nocturnal increases in tissue malic acid content. The species of *Talinum* are distinguished primarily by floral characters, as differences in vegetative morphology are difficult to differentiate (Murdy and Carter, 1985; Great Plains Flora Association, 1986). Cytotaxonomic work on these species has suggested that *T. teretifolium* is a putative allotetraploid derivative of two of the other species (*T. parviflorum* and *T. mengesii*; Carter and Murdy, 1985; Murdy and Carter, 1985). Phylogenetic relationships among the other species in this study are unknown. Nevertheless, given the high degree of morphological similarity among these species, particularly in leaf structure, some useful comparisons of photosynthesis may be made among plants differing in ploidal level. Therefore, the purpose of this study was to determine if ploidal level and the degree of CAM were correlated in several populations of five species of *Talinum*. The correlations of ploidal level and CAM acid fluctuations with tissue water content (fresh weight/dry weight), as an estimate of succulence, were also examined.

MATERIALS and METHODS

Growth conditions—Plants from 23 populations of five species of *Talinum* were collected during June and July 1987 and transferred to the University of Kansas greenhouse. The plants were potted in standard greenhouse soil and watered three times per week until all plants were dormant in September 1987. The plants were then cold-treated in a refrigerator (4°C) for 3.5 mo, and then resprouted in a growth chamber where they were watered daily with deionized water and fertilized once per wk. Growth under these conditions appeared vigorous, and all plants were mature and flowering when analyzed. For further information regarding growth conditions, soil, and fertilizer, see Harris and Martin (1991).

Malic acid and fresh weight to dry weight ratio determinations—After 65 d of growth in the growth chamber, four to five plants from each population were sampled for malic acid concentration by removing one shoot with ten to 15 leaves 0.5 hr before lights-on and then removing the remaining shoot material 0.5 hr before lights-out. The shoots were frozen immediately and stored at -65°C until they were assayed for malic acid content by enzymatic analysis (Gutmann and Wahlefeld, 1974). Fresh weight was obtained for the same leaf tissue assayed for malic acid content by weighing the tissue prior to the assay. Malic acid concentration was determined in sap expressed from the leaf tissue by centrifuging the leaf sample in a syringe barrel fitted with a filter paper disc, according to the method described in Smith and Lüttge (1985). The dry weight of the remaining tissue was then obtained after drying for 3 d at 65°C. Shoot fresh weight to dry weight ratio (FW/DW), an estimate of tissue succulence, was calculated for leaf samples that were collected before lights-on in the growth chamber when leaves were fully hydrated.

Chromosome counts—Ploidal levels of plants from the study populations of the three eastern species (*T. calcaricum*, *T. mengesii*, and *T. teretifolium*) were determined from root tip squashes according to the procedure of Car-

ter and Murdy (1985). Root tissue was taken from plants obtained in the field as well as from intraspecific crosses between plants of known ploidal level (Carter and Murdy, 1987). Plants for chromosome counts of *T. calycinum* and *T. parviflorum* were collected from the field during the summer of 1990 and transferred to the greenhouse where they were potted in soil, watered daily, and fertilized once per week as described above. After 1 mo of growth in the greenhouse, plants were uprooted, and all roots were trimmed from the rhizomes. The rhizomes were then wrapped in moist paper towels and placed in a growth chamber for 3 d. Growth chamber conditions were: 13-hr photoperiod with day/night temperatures of 25/20°C and day/night vapor pressure deficits of 1.6/0.7 kPa. The photosynthetic photon flux density (PPFD) at plant height was 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

After 3 d, new roots were removed from the rhizomes 1 to 2 hr after lights-on and placed in a saturated aqueous solution of 1-bromonaphthalene for 2 hr. The roots were then rinsed in distilled water and fixed overnight in Farmer's solution (ethanol : glacial acetic acid [3:1, v/v]). The roots were then immersed in an ethanol : concentrated hydrochloric acid (1:1, v/v) solution for 5–6 min, rinsed in deionized water, teased apart on a slide, and stained in a carbol fuchsin stain (Z.-R. Wang, personal communication). A coverslip was then applied, and the root tips were thumb-squashed. Chromosome counts were made on cells from three to five root tips per individual, three individuals per study population.

Statistical analysis—Because overall means of malic acid accumulations and FW/DW ratios for diploid and tetraploid plants were heteroscedastic, they were analyzed for significant differences using a Mann-Whitney *U*-test. Average values for malic acid fluctuation and FW/DW for plants grouped by species and ploidal level were analyzed for significant differences with a Kruskal-Wallis test. Statistically significant ($P < 0.05$) means were analyzed using a multiple comparison-of-means test based on average ranks in the Kruskal-Wallis test (Daniel, 1978). Correlation coefficients were used to examine the relationship between malic acid fluctuation and FW/DW, and were tested for significance using the procedure of Sokal and Rohlf (1981). All analyses were performed with the Statview 512+ statistical package (Brainpower Inc., Ventura, CA).

RESULTS

As reported previously, the *Talinum mengesii* populations were diploid ($2n = 24$), whereas *T. calcaricum* and *T. teretifolium* were tetraploid ($2n = 48$; Table 1). The individuals examined in the two midwestern species were diploid except for one tetraploid population of *T. parviflorum* and two tetraploid populations of *T. calycinum* (Table 1).

In a comparison of average malic acid fluctuations among plants grouped by species and ploidal level, two tetraploid groups, *T. calcaricum* and *T. calycinum*, had significantly higher malic acid fluctuations than the other groups (Table 2). On the other hand, the tetraploid individuals of *T. parviflorum* and *T. teretifolium*, as well as all diploid populations (*T. calycinum*, *T. mengesii*, and

TABLE 1. Population, study site location, rock outcrop type, and ploidal level in root tip cells for individuals ($N = 3$) from 23 populations of five species of *Talinum*

Species-population	Study site	Outcrop type	Chromosome number	Reference ^a
<i>T. mengesii</i> -1	Gadsden, AL	Sandstone	24	Murdy and Carter, 1985
<i>T. mengesii</i> -2	Laochapoka, AL	Granite	24	Murdy and Carter, 1985
<i>T. mengesii</i> -3	New Georgia, GA	Granite	24	Murdy and Carter, 1985
<i>T. mengesii</i> -4	Hamilton, AL	Conglomerate	24	Murdy and Carter, 1985
<i>T. mengesii</i> -5	Loganville, GA	Granite	24	Murdy and Carter, 1985
<i>T. calcaricum</i> -1	Chapel Hill, TN	Limestone	48	Carter and Murdy, 1987
<i>T. calcaricum</i> -2	Pulaski, TN	Limestone	48	Carter and Murdy, 1987
<i>T. calcaricum</i> -3	Newburg, AL	Limestone	48	Carter and Murdy, 1987
<i>T. calcaricum</i> -4	Gladeville, TN	Limestone	48	Krebs, 1971
<i>T. teretifolium</i> -1	Gasburg, VA	Granite	48	^b
<i>T. teretifolium</i> -2	Rockdale, GA	Granite	48	Carter and Murdy, 1985
<i>T. teretifolium</i> -3	Goshenville, PA	Serpentine	48	^b
<i>T. teretifolium</i> -4	Sandersville, GA	Sandstone	48	Carter and Murdy, 1985
<i>T. teretifolium</i> -5	Hiddenite, NC	Granite	48	^b
<i>T. parviflorum</i> -1	Warrenton, MO	Granite	24	This study
<i>T. parviflorum</i> -2	Kanopolis, KS	Sandstone	24	This study
<i>T. parviflorum</i> -3	Norden, NE	Gravel bar	24	This study
<i>T. parviflorum</i> -4	Brady, TX	Granite	24	This study
<i>T. parviflorum</i> -5	Kersey, CO	Gravel bar	48	This study
<i>T. calycinum</i> -1	Farmington, MO	Sandstone	48	This study
<i>T. calycinum</i> -2	Pioneer, MO	Chert	48	This study
<i>T. calycinum</i> -3	Kanopolis, KS	Sandstone	24	This study
<i>T. calycinum</i> -4	Norden, NE	Gravel bar	24	This study

^a References indicate sources of chromosome data.

^b Estimated from comparisons of floral and electrophoretic characters with plants of known ploidal level.

T. parviflorum) exhibited low to intermediate levels of malic acid fluctuation. Individuals of *T. parviflorum* had the lowest malic acid fluctuations and showed no differences between ploidal levels. Conversely, the tetraploid individuals of *T. calycinum* had significantly higher average malic acid fluctuations than the diploid plants of the same species (Table 2). Diploid individuals of all species exhibited malic acid fluctuations below $155 \mu\text{mol g}^{-1}$ DW except for two individuals of *T. calycinum*, whereas nearly all tetraploid individuals of *T. calycinum* and *T. calcaricum* had malic acid fluctuations above $155 \mu\text{mol g}^{-1}$ DW. Overall, nocturnal accumulations of malic acid were significantly greater in tetraploid plants than in diploid individuals (Table 2).

Malic acid fluctuation was positively correlated with FW/DW when all individuals were examined together (Fig. 1). Within species-ploidal groups, however, malic acid fluctuation was significantly correlated with FW/DW

only for *T. mengesii* (Fig. 1). Tetraploid plants, when examined together, exhibited a higher FW/DW than diploid plants (Table 2).

DISCUSSION

Tetraploid plants of *T. calycinum* and *T. calcaricum* had significantly higher levels of CAM acid fluctuations than the other plants, which supports the hypothesized relationship between ploidal level and CAM. In fact, when all plants were examined according to ploidal level, the degree of CAM was greater in tetraploids than diploids. Some tetraploids, however, did not exhibit greater accumulations of malic acid than their diploid congeners. Thus, in the case of *T. teretifolium* and *T. parviflorum*, polyploidy has not resulted in increased malic acid fluctuations, relative to their putative progenitors.

Overall, the degree of CAM increased with increasing

TABLE 2. Average day/night malic acid fluctuation and average FW/DW for all individuals ($N = 4$ or 5 per population) of a given ploidal level within five species of *Talinum* grown under well-watered conditions in a growth chamber

Species	Chromosome number	Number of populations	Number of individuals	FW/DW ^{a,b}	Δ malate ^{a,b}
<i>T. mengesii</i>	24	5	21	13.4 (0.6) a	$95.8 (7.8) \text{ a,b}$
<i>T. calcaricum</i>	48	4	17	16.7 (0.6) b	$208.2 (20.4) \text{ c}$
<i>T. teretifolium</i>	48	5	22	11.9 (0.4) a	$127.9 (12.0) \text{ b}$
<i>T. parviflorum</i>	24	4	19	13.2 (0.5) a	$83.2 (6.8) \text{ a}$
<i>T. parviflorum</i>	48	1	4	13.0 (1.1) a	$85.1 (21.9) \text{ a}$
<i>T. calycinum</i>	24	2	9	13.2 (0.6) a	$145.3 (22.9) \text{ b}$
<i>T. calycinum</i>	48	2	10	15.6 (0.7) b	$235.1 (21.2) \text{ c}$
All diploid plants	24	11	49	13.3 (0.6)	$95.4 (6.6)$
All tetraploid plants	48	12	53	14.3 (0.9)*	$167.5 (11.5) \text{ ***}$

^a Standard errors of the means are given in parentheses.

^b Different letters following group means denote significantly different means at $P < 0.05$, according to a Kruskal-Wallis test and a nonparametric multiple comparison-of-means test. Overall means compared with a Mann-Whitney U -test (* = $P < 0.05$; *** = $P < 0.001$).

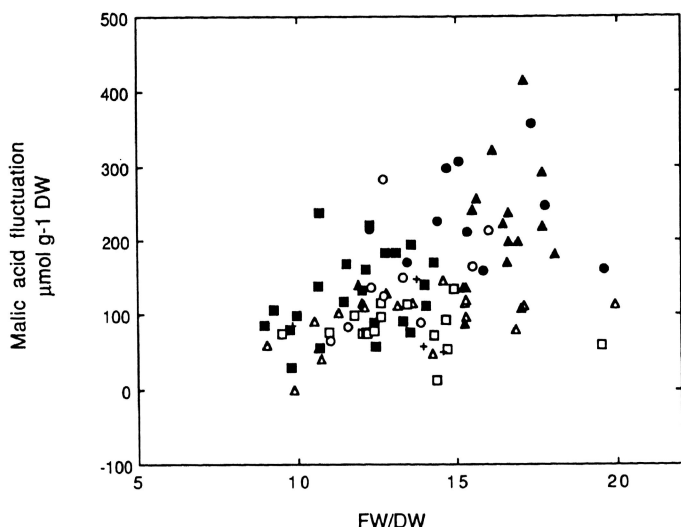


Fig. 1. Relationship between malic acid fluctuation and FW/DW (a.m. values) for 102 individuals of five species of *Talinum* grown under well-watered conditions in a growth chamber. Correlation coefficient (r) for all individuals grouped together = 0.46**. Symbols, species, ploidal level, and correlation coefficients are: open triangles, *T. mengesii*, $2n = 24$, $r = 0.48^*$; closed triangles, *T. calcaricum*, $2n = 48$, $r = 0.40$, not significant (ns); closed squares, *T. teretifolium*, $2n = 48$, $r = 0.36$, ns; open squares, *T. parviflorum*, $2n = 24$, $r = -0.13$, ns; crosses, *T. parviflorum*, $2n = 48$, $r = -0.12$, ns; open circles, *T. calycinum*, $2n = 24$, $r = 0.47$, ns; closed circles, *T. calycinum*, $2n = 48$, $r = 0.04$, ns. * and ** denote $P < 0.05$ and 0.01 , respectively.

leaf succulence, similar to results obtained previously for several species of *Sedum* (Teeri, Tonsor, and Turner, 1981). This conclusion is based on the assumption that the FW/DW of the leaf tissue is an accurate estimate of leaf succulence and perhaps cell volume. These findings support the expected relationship between cell volume and degree of CAM given considerations of vacuolar limitations on nocturnal CO_2 uptake (Winter, 1985).

In summary, the results of this study suggest that tetraploidy is correlated with greater levels of CAM in *Talinum*. A greater degree of CAM may substantially increase the persistence of plants in frequently drought-stressed habitats such as rock outcrops, as higher levels of CAM may effect lower rates of daytime transpiration and increased resilience during long-term drought (Harris and Martin, 1991).

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