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Ploidy Effects in Isogenic Populations of Alfalfa[1]

I. RIBULOSE-1,5-BISPHOSPHATE CARBOXYLASE, SOLUBLE PROTEIN, CHLOROPHYLL, AND DNA IN LEAVES

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ABSTRACT

The influence of polyploidization on ribulose-1,5-bisphosphate carboxylase (RuBPCase), buffer-soluble protein (BSP), chlorophyll (Chl), and DNA was examined in fully expanded leaves of isogenic diploid-tetraploid (DDC 2X-4X) and tetraploid-octoploid (IC 4X-8X) sets of alfalfa (*Medicago sativa* L.). The concentration of RuBPCase in leaf extracts was determined by rocket immunoelectrophoresis. Activities of RuBPCase, expressed per milligram protein or per milligram Chl, and leaf tissue concentrations of RuBPCase, BSP, Chl, and DNA were similar between ploidy levels of the DDC 2X-4X set. Tetraploids and octoploids were similar in RuBPCase activities, expressed per milligram protein or per milligram Chl, and in leaf tissue concentrations of RuBPCase and DNA. Octoploids were significantly lower than tetraploids in concentrations of Chl and BSP.

When compared on a per leaf basis, tetraploids were 80% higher in BSP and essentially double comparable diploids in fresh weight, RuBPCase, Chl, and DNA. The observation that leaves of the DDC tetraploid population contain twice as much DNA as comparable diploids suggests that leaves of both ploidy levels contain similar numbers of cells. Leaves of the octoploid population were 33% to 80% higher than corresponding tetraploids in BSP, fresh weight, RuBPCase, Chl, and DNA. Ratios of RuBPCase to DNA and Chl to DNA were similar across ploidy levels of both isogenic sets suggesting that cellular content of Chl and RuBPCase increases proportionately with the amount of DNA per cell.

Polyploidization has played an important role in plant evolution. Increases in nuclear ploidy have been reported to affect net photosynthesis (5, 7, 12, 22), transpiration (5, 26), enzyme activities (1, 7, 8, 22, 29), photosynthetic electron transport (29), and isozyme expression (7, 30).

RuBPCase[3]/oxygenase (EC 4.1.1.39) catalyzes the carboxylation of RuBP to form two molecules of glycerate 3-P and the oxygenation of RuBP to form one molecule each of glycerate 3-P and glycolate 2-P. Increased RuBPCase activity, caused by either increased amount of enzyme or level of enzyme activation, may result in higher net photosynthetic rates (11, 12, 14, 20, 22). Randall *et al.* (22) reported that a decaploid tall fescue (*Festuca arundinacea* Schreb.) genotype had both a higher CER and a RuBPCase specific activity 1.3- to 2-fold greater than hexaploid genotypes. Increased synthesis of RuBPCase in the decaploid tall fescue genotype was suggested to be partially responsible for the high CER. In naturally occurring allopolyploids (4X, 6X, 8X, and 10X) of tall fescue, CER increased significantly with ploidy (5, 12). Ploidy, however, had no significant effect on specific activity of fully activated RuBPCase (12), photosynthetic electron transport, or photophosphorylation (13) in tall fescue. Leaf concentrations of RuBPCase have been reported to increase with ploidy in tall fescue (12) but they remained constant in isogenic diploid and tetraploid ryegrass (*Lolium perenne* L.) (7, 24).

In a colchicine-derived euploid series of castor bean (*Ricinus communis* L.), increases in nuclear ploidy were associated with increased chloroplast RuBPCase specific activities but decreased photosynthetic rates of leaves, chloroplast O_2 evolution, and electron transport (29). Setter *et al.* (26) reported that ploidy had little or no effect on CER, leaf area, or leaf weight in colchicine-derived 2X-4X and 4X-8X alfalfa (*Medicago sativa* L.). Restoration of heterozygosity by hybridization at the 2X and 4X alfalfa ploidy levels resulted in tetraploids with greater leaf areas, transpiration per leaf, and CER per leaf than in corresponding diploids (26). When expressed per unit leaf area, CER and transpiration, however, were unchanged with polyploidization in alfalfa (26).

Elucidating the effects of nuclear ploidy on the genetic expression of enzymes has been difficult due to limitations of most plant material used in previous studies. Colchicine-induced polyploids have been associated with inbreeding depression equivalent to between 2.2 and 3.8 generations of selfing (3). Decreased photosynthetic rates with increased nuclear ploidy have been attributed to such inbreeding depression (29). Genetic differences between naturally occurring allopolyploids may confound the consequences of ploidy effects. To avoid the limitations of inbreeding depression and genetic differences between ploidy levels, isogenic diploid-tetraploid and tetraploid-octoploid alfalfa populations, to which heterozygosity was restored, were used in this study. These isogenic populations have equivalent hybrid states and the same genes and gene frequencies (21). This study was conducted to assess the effects of ploidy level on RuBPCase, soluble protein, Chl, and DNA in alfalfa leaves.

MATERIALS AND METHODS

Plant Material and Culture. The derivations of isogenic diploid-tetraploid (DDC 2X-4X) and tetraploid-octoploid (IC 4X-8X) populations of alfalfa (*Medicago sativa* L.) with equivalent gene frequencies and hybrid states have been described (21). Plants were grown in plastic pots (20 × 20 × 30 cm with 10 drainage holes in the bottom) filled with 10 L of a 1:1 sand to soil mixture.

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[3] Abbreviations: RuBPCase, ribulose-1,5-bisphosphate carboxylase; RuBP, ribulose-1,5-bisphosphate; CER, CO_2 exchange rate; BSP, buffer-soluble protein; IgG, gamma immunoglobulin.

Each pot contained two plants and received 400 ml distilled H_2O daily. Twice-a-week pots received 400 ml nutrient solution instead of water. The nutrient solution (pH 6.5) contained 0.5 mM KH_2PO_4, 1 mM $MgSO_4$, 125 μM K_2SO_4, 0.71 mM $CaCl_2$, 7.5 mM KNO_3, 9.1 μM $MnCl_2$, 22.7 μM H_3BO_3, 0.017 μM $CoSO_4$, 0.20 μM $ZnSO_4$, 0.21 μM $(NH_4)_6Mo_7O_{24}$, 0.079 μM $CuSO_4$, and 0.05 g sodium ferric diethyl pentaacetate/l (approximately 90 μM Fe, Sequestrene 330 Fe; Ciba-Geigy, Ardsley, NY). Photosynthetic photon flux density at pot top level in the growth chamber was 500 $\mu E \cdot m^{-2} \cdot s^{-1}$ as provided by a mixture of fluorescent and incandescent lamps. Air temperature during the 16-h photoperiod was 28°C. The temperature during the 8-h dark period was 21°C. The RH was maintained at about 60%. Plants in the greenhouse, grown during the first 2 months of 1981, received 16 h of supplemental lighting supplied by a mixture of fluorescent and incandescent lamps. Greenhouse temperatures ranged from 20 to 27°C. Plants were cut to within 5 cm of the crown every 4 weeks. Fresh and dry weights of leaves and stems were measured for two cuttings of both greenhouse- and growth chamber-grown plants after 4 weeks of regrowth.

Purification of RuBPCase. RuBPCase was extracted and purified from leaves of each ploidy level of both isogenic alfalfa populations according to Hall and Tolbert (9). Further purification was performed by solubilizing the ammonium sulfate-precipitated protein in 2 ml buffer (50 mM Tricine, pH 8.0 at 4°C, and 10 mM β-mercaptoethanol) and centrifugation for 35 h at 82,500*g* through a linear 0.29 to 0.88 M sucrose gradient containing 25 mM Tris (pH 7.5 at 4°C), 25 mM $MgCl_2$, 25 mM KCl, and 5 mM DTT. The gradient was fractionated and scanned at 280 nm. The RuBPCase-containing fraction (3 ml) was applied to a Sephadex G-100 column (1.5 × 80 cm) equilibrated with 50 mM Tris (pH 7.6 at 4°C) and 10 mM β-mercaptoethanol, and the void volume was collected. The eluted protein was precipitated in 60% $(NH_4)_2SO_4$, pH 7.6, and stored at −15°C. The enzyme preparations appeared to be homogeneous as determined by polyacrylamide gel electrophoresis.

Preparation of RuBPCase-Specific Immunoglobulins. Four female New Zealand albino rabbits were used for the production of immunoglobulins against RuBPCase isolated from each ploidy level of both isogenic sets. Purified RuBPCase, 2 mg in 1 ml of 0.15 M NaCl, and 20 mM phosphate (pH 7.5), was mixed 1:1 with Freund's complete adjuvant and emulsified. Emulsions were injected subcutaneously at eight sites on the shaved backs of rabbits. After one month, similar injections were given. Two weeks later, blood (up to 25 ml) was collected biweekly from the marginal ear vein of each rabbit. Booster injections of RuBPCase emulsified with incomplete adjuvant were given every 6 months. The collected blood was allowed to clot at 20°C for 1 h and then left at 4°C for 16 h. Sera were centrifuged at 5,000*g* for 20 min and the supernatants were stored at −15°C. The IgG fraction was purified on DEAE Sephadex A50 according to Harboe and Ingild (10) and stored in 0.1 M NaCl and 15 mM NaN_3 at 4°C.

Extraction and Assay of RuBPCase and Chemical Constituents. Fully expanded trifoliolate leaves (from the 3rd to 6th nodes from the apex on plants 4 weeks after cutting) were used for extraction of DNA, Chl, BSP, and RuBPCase. Extractions were performed at 4°C 6 h after the start of the photoperiod. Leaves (1 g) were counted, washed, and then homogenized in 9 ml of buffer (100 mM Tris [pH 8.6 at 5°C], 20 mM $MgCl_2$, 10 mM $NaHCO_3$, 1 mM DTT with 0.2 g insoluble PVP) in a VirTis model 60K homogenizer (1 min at 30,000 rpm). Homogenates were filtered through a 500-μm mesh nylon screen. Duplicate 0.5-ml aliquots of the homogenate were mixed with 2 ml of 2.5 M NaCl, 10 mM Tris (pH 7.0 at 25°C), and 10 mM EDTA for the DNA assay. Duplicate 0.2-ml aliquots of the homogenate were added to 4.8 ml of 95% (v/v) ethanol for Chl determination. The remaining homogenate was centrifuged at 27,000*g* for 25 min at 4°C. Duplicate 20-μl aliquots of the supernatant were used for the RuBPCase activity assay. The remaining supernatant was diluted 1:10 with 0.15 M NaCl, 20 mM phosphate (pH 7.8), and 15 mM NaN_3 for determination of concentrations of BSP and RuBPCase.

DNA concentration was determined by the quantitative fluorometric method of Baer *et al.* (2). Chl concentration was estimated using the constants of Wintermans and DeMots (32). Concentration of BSP was determined as described by Bradford (4) as using BSA as the standard.

RuBPCase concentration in leaf extracts was quantitated by rocket immunoelectrophoresis (31). A 35-ml solution containing 0.9% (w/v) agarose, 40 mM barbital (pH 8.6), 5 mM NaN_3, and 700 μg anti-RuBPCase IgG was poured onto a glass plate (205 × 110 × 1.5 mm) heated to 40°C. The plate was cooled to 25°C for 10 min. Circular wells, 4 mm in diameter, were punched into the gel. The anode-cathode buffer contained 30 mM barbital (pH 8.6) and 4 mM NaN_3. With a potential of 70 v across the gel, 10-μl samples were applied to each well. Electrophoresis was performed at 90 v for 8 to 10 h at 12°C. The gel was pressed, dried, and stained with Coomassie blue G to visualize the rockets. Regression equations were derived for each standard curve ($r > 0.97$).

RuBPCase activity was assayed as described by Lorimer *et al.* (15). Duplicate 20-μl aliquots of the centrifuged homogenate were incubated (25°C for 30 min) with 0.5 ml of 100 mM Tris (pH 8.2 at 25°C), 20 mM $MgCl_2$, 5 mM DTT, and 20 mM $NaH^{14}CO_3$ (0.2 $\mu Ci \cdot \mu mol^{-1}$) to allow for CO_2/Mg^{2+} activation of RuBPCase. The reaction was initiated by the addition of 10 μl of 20 mM RuBP and terminated after 1.5 min by the addition of 120 μl of 2 N HCl. Backgrounds were determined by including zero reaction time samples and samples without RuBPCase or RuBP. A portion of the assay volume, 500 μl, was transferred to a scintillation vial, dried and counted in 10 ml of 75% (v/v) xylene, 25% (v/v) Triton X-114, and 6 g PPO/l with a scintillation spectrometer.

Experimental Analysis. Each experiment consisted of two extractions from each ploidy level of the DDC and IC sets. Experiments were repeated two times and four times for greenhouse- and growth chamber-grown plants, respectively. A two-factor analysis of variance, with ploidy level and location as main effects, was computed for each isogenic set using the Statistical Analysis System (SAS) software package (SAS Institute Inc., Raleigh, NC).

Table I. *Effect of Ploidy on Fresh and Dry Weights of Leaves and Stems of Alfalfa Harvested after Four Weeks of Regrowth*

Parameter	Ploidy Level			
	DDC 2×	DDC 4×	IC 4×	IC 8×
Fresh wt leaves (g/plant)	13.1 ± 0.8[a]	20.3 ± 1.5	24.8 ± 3.0	24.7 ± 2.2
Fresh wt stems (g/plant)	11.6 ± 0.7	16.7 ± 1.2	18.7 ± 2.3	18.3 ± 1.6
Dry wt leaves (g/plant)	2.8 ± 0.2	4.1 ± 0.3	5.2 ± 0.6	5.1 ± 0.4
Dry wt stems (g/plant)	2.4 ± 0.3	3.3 ± 0.2	3.9 ± 0.5	3.3 ± 0.3
% Moisture in shoots	79.0	79.8	79.1	81.6

[a] Mean ± SE.

RESULTS

Plants of the DDC 4X population were approximately 40 to 50% greater than those of 2X population in fresh and dry weights of leaves and stems (Table I). Plants of the IC 4X-8X populations were similar in fresh and dry weights of leaves and stems. Moisture percentages of shoots were similar between ploidy levels of both isogenic sets.

When expressed per trifoliolate leaf, tetraploids were essentially double diploids in fresh weight, DNA, Chl, and RuBPCase (Table II). Leaves of tetraploids were 80% higher in BSP than were comparable diploids. In the DDC 2X-4X set, leaves of greenhouse-grown plants were significantly higher ($P < 0.05$) in fresh weight and BSP than were leaves of growth chamber-grown plants.

Fresh weight and the content of DNA and Chl per trifoliolate leaf of the IC 8X population were 50 to 80% greater than in comparable tetraploids (Table III). All differences in the above parameters were highly significant ($P < 0.01$). Octoploid leaves of greenhouse-grown plants exceeded comparable tetraploids by 50% and 70% in content of BSP and RuBPCase, respectively, whereas growth chamber octoploids were only 33% greater than corresponding tetraploids in these parameters.

Chl *a* to *b* ratios and leaf tissue concentrations of Chl, DNA, BSP, and RuBPCase were not significantly different between diploids and tetraploids (Table IV). Chl *a* to *b* ratios, however, were significantly lower ($P < 0.05$) in greenhouse-*versus* growth chamber-grown plants of the DDC 2X-4X set. Leaf DNA concentrations of growth chamber plants were slightly higher than those for greenhouse-grown plants. Ratios of BSP to DNA were similar between ploidy levels of growth chamber plants, whereas greenhouse-grown diploids had a significantly higher ($P < 0.05$) BSP to DNA ratio than did comparable tetraploids. Ploidy appeared to have little effect on ratios of Chl to DNA, RuBPCase to BSP, RuBPCase to Chl, and RuBPCase to DNA for the DDC 2X-4X set (Table IV). Ploidy had no effect on RuBPCase activity when expressed per mg BSP or per mg Chl in the DDC 2X-4X set. RuBPCase activity expressed per mg RuBPCase was significantly lower ($P < 0.05$) in tetraploids compared to diploids. The concentrations of RuBPCase per mg BSP and RuBPCase activity per mg BSP were lower ($P < 0.01$ and $P < 0.05$, respectively) in greenhouse plants than in growth chamber plants.

The Chl concentration of octoploid leaves was significantly lower ($P < 0.05$) than that of tetraploids (Table V). In the IC 4X-8X set, growth chamber plants had significantly higher ($P < 0.01$) Chl *a* to *b* ratios than did greenhouse-grown plants. Ploidy had little effect on DNA concentration and ratios of Chl to DNA, RuBPCase to BSP, and RuBPCase to Chl (Table V). Leaf DNA concentrations of growth chamber plants were higher ($P < 0.05$) than those for greenhouse-grown plants. Octoploids were significantly lower in BSP concentration ($P < 0.01$) and the ratio of BSP to DNA ($P < 0.05$) than were comparable tetraploids. Growth chamber tetraploids were higher in RuBPCase concentration ($P < 0.01$) and in the ratio of RuBPCase to DNA ($P < 0.05$) than were comparable octoploids, whereas greenhouse tetraploids and octoploids were similar in these parameters. RuBPCase activity expressed per mg BSP or per mg Chl was not significantly different between ploidy levels of the IC 4X-8X set. RuBPCase activity expressed per mg RuBPCase was significantly lower ($P < 0.01$) in octoploids compared to tetraploids. Greenhouse-grown plants were higher than growth chamber plants in BSP per mg DNA ($P < 0.05$) and RuBPCase activity per mg RuBPCase ($P < 0.01$), but lower in RuBPCase concentration ($P < 0.05$) and RuBPCase per mg BSP ($P < 0.01$).

DISCUSSION

Polyploid evolution is a complex process that is dependent on chromosome doubling, heterozygosity, mutation, and regularization of chromosome-pairing (3, 27). Study of the physiological and biochemical effects of polyploidization as an individual process of genome duplication requires plant material which differs only in chromosome number. The isogenic DDC 2X-4X and IC 4X-8X alfalfa populations used in this study have equivalent hybrid states and the same genes and gene frequencies.

Pfeiffer *et al.* (21) reported that net CO_2 exchange per plant was equivalent across ploidy levels in the isogenic DDC 2X-4X and IC 4X-8X alfalfa populations. They observed no significant differences between ploidy levels in fresh and dry weights of leaves, stems, and roots; root respiration; nodule number; nodule weight; and acetylene reduction. This lack of response to ploidy can be attributed to growth of two plants in 1 L of rooting medium in the previous study (21). Growth of the DDC 2X-4X and IC 4X-8X alfalfa in 10 L of rooting medium in the present study resulted in DDC 4X plants that were 40% higher than comparable diploids in fresh and dry weights of leaves and stems whereas tetraploids and octoploids were similar in these parameters. In addition, fresh and dry weights of leaves and stems per plant were between 4 and 7 times greater for plants grown in 10 L of rooting medium than for the same genotypes grown in 1-L containers (21).

In the present study, the effects of ploidy on fresh weight per leaf were similar to those observed with field-grown alfalfa (6). Tetraploid leaves were essentially double comparable diploids in fresh weight, Chl, RuBPCase, and DNA. The observation that tetraploid leaves contain twice as much DNA as diploids suggests that leaves of both ploidy levels contain similar numbers of cells. In fact, DNA per protoplast was observed to double in the tetraploid as compared to the diploid (18). A doubling in ploidy apparently did not result in a doubling in all soluble proteins

Table II. *Effects of Ploidy Level and Location on Fresh Weight, DNA, Chl, BSP, and RuBPCase per Fully Expanded Trifoliolate Leaves (3rd to 6th Nodes from the Apex)*

Parameter	Ploidy				Treatment Effects		
	Greenhouse-grown plants		Growth chamber-grown plants				
	DDC 2X	DDC 4X	DDC 2X	DDC 4X	P[a]	L[b]	P × L
	trifoliolate leaf^{-1}						
Fresh wt (mg)	28.8	63.5	23.4	47.6	**[c]	*	NS
DNA (μg)	19.3	43.3	19.4	35.7	**	NS	NS
Chl (μg)	86.4	170.2	70.3	146.6	**	NS	NS
BSP (mg)	1.4	2.5	1.0	1.8	**	*	NS
RuBPCase (mg)	0.6	1.2	0.5	1.0	**	NS	NS

[a] Ploidy.
[b] Location (greenhouse *versus* growth chamber).
[c] *, ** denote significance at the 0.05 and 0.01 level, respectively.

Table III. *Effects of Ploidy Level and Location on Fresh Weight, DNA, Chl, BSP, and RuBPCase per Fully Expanded Trifoliolate Leaves (3rd to 6th Nodes from the Apex)*

Parameter	Ploidy				Treatment Effects		
	Greenhouse-grown plants		Growth chamber-grown plants				
	IC 4X	IC 8X	IC 4X	IC 8X	P[a]	L[b]	P × L
	trifoliolate leaf^{-1}						
Fresh wt (mg)	57.5	101.4	52.0	85.9	**[c]	NS	NS
DNA (μg)	38.5	67.2	40.5	62.9	**	NS	NS
Chl (μg)	166.0	270.9	170.6	252.0	**	NS	NS
BSP (mg)	2.4	3.6	2.1	2.8	**	NS	NS
RuBPCase (mg)	1.0	1.7	1.2	1.6	**	NS	NS

[a] Ploidy.
[b] Location.
[c] *, ** denote significance at the 0.05 and 0.01 level, respectively.

Table IV. *Effects of Ploidy Level and Location on Chl, DNA, BSP, and RuBPCase from Fully Expanded Trifoliolate Leaves (3rd to 6th Nodes from the Apex)*

Parameter	Ploidy				Treatment Effects		
	Greenhouse-grown plants		Growth chamber-grown plants				
	DDC 2X	DDC 4X	DDC 2X	DDC 4X	P[a]	L[b]	P × L
Chl concn (mg/g fresh wt)	2.99	2.67	2.99	3.17	NS	NS	NS
Chl *a*:*b* ratio	3.28	3.46	3.62	3.49	NS	*[c]	*
DNA concn (μg/g fresh wt)	660	680	840	761	NS	NS	NS
Chl/DNA (mg/mg)	4.66	3.94	3.67	4.21	NS	NS	NS
BSP concn (mg/g fresh wt)	47.3	40.0	40.5	37.7	NS	NS	NS
BSP/DNA (mg/mg)	73.9	58.8	50.0	51.1	*	NS	NS
RuBPCase concn (mg/g fresh wt)	21.0	18.2	19.4	21.1	NS	NS	NS
RuBPCase/BSP (mg/mg)							
RuBPCase/Chl (mg/mg)	0.44	0.45	0.48	0.56	NS	**	NS
RuBPCase/DNA (mg/mg)	6.99	6.82	6.42	6.72	NS	NS	NS
RuBPCase activity	32.6	26.8	24.0	28.2	NS	NS	NS
nmol CO_2/min·mg BSP	369	356	404	414	NS	*	NS
μmol CO_2/hr·mg Chl	350	320	367	345	NS	NS	NS
nmol CO_2/min·mg RuBPCase	834	779	853	757	*	NS	NS

[a] Ploidy.
[b] Location.
[c] *, ** denote significance at the 0.05 and 0.01 level, respectively.

because BSP per leaf in tetraploids was only 80% higher than in comparable diploids.

Concentrations of Chl, DNA, and BSP in leaf tissue were not significantly different in any DDC 2X-4X comparison (Table IV). These results are consistent with data of field-grown 2X-4X alfalfa (6). Concentrations of soluble protein in leaves have been shown to be similar across ploidy levels in other plant species (12, 24, 28), and Tal (28) reported that DNA concentrations of diploid and autotetraploid tomato (*Lycopersicon esculentum*) leaves were not significantly different.

The lower concentrations of Chl and BSP in octoploids compared to tetraploids (Table V) and the failure of octoploids to double the leaf parameters of tetraploids (Table III) may be attributed to aneuploidy in the octoploid population, limitations of maximum heterozygosity, or the exceeding of an optimal ploidy level. Agriculturally important cultivars of alfalfa are primarily tetraploids. Considerable variation of somatic chromosome numbers has been observed among progenies of alfalfa at ploidy levels greater than 4X. Yen and Murphy (33) reported that the intercrossing of hexaploid alfalfa (*Medicago spp.*) resulted in an F_1 population in which hexaploids ($2n = 48$) constituted approximately 64% of the population and chromosome numbers ranged from $2n = 36$ to $2n = 51$. The IC 4X-8X alfalfa populations were derived from twelve tetraploid selections which had spontaneously doubled in tissue culture (21). To develop the isogenic 4X-8X populations with heterozygosity restored in the 8X population, twelve plants at each ploidy level were randomly intercrossed. Although chromosome number probably varied in the 8X population, the expression of data on a DNA basis allowed a correction for this variability. Maximum heterozygosity, or the highest frequency of tetraallelic loci in tetraploids, has been proposed to be a requirement for full fertility and vegetative vigor in alfalfa (3). The frequency of octo-allelism in the octoploid population may have been insufficient for maximum heterosis. Many plant species have an optimal ploidy level beyond which vigor and viability decreases (19).

The effect of gene dosage, nuclear ploidy, on the gene product RuBPCase was examined in the DDC 2X-4X and IC 4X-8X populations. Rocket immunoelectrophoresis was used to quantitatively determine total RuBPCase protein. Ratios of RuBPCase

Table V. *Effects of Ploidy Level and Location on Chl, DNA, BSP, and RuBPCase from Fully Expanded Trifoliolate Leaves (3rd to 6th Nodes from the Apex)*

Parameter	Ploidy				Treatment Effects		
	Greenhouse-grown plants		Growth chamber-grown plants				
	IC 4X	IC 8X	IC 4X	IC 8X	P[a]	L[b]	P × L
Chl concn (mg/g fresh wt)	2.89	2.65	3.26	2.97	*[c]	**	NS
Chl *a*:*b* ratio	3.36	3.35	3.54	3.51	NS	**	NS
DNA concn (μg/g fresh wt)	665	657	783	736	NS	*	NS
Chl/DNA (mg/mg)	4.37	4.07	4.23	4.12	NS	NS	NS
BSP concn (mg/g fresh wt)	42.1	35.2	41.2	31.9	**	NS	NS
BSP/DNA (mg/mg)	63.7	54.0	53.7	44.3	*	*	NS
RuBPCase concn (mg/g fresh wt)	17.2	17.1	23.8	19.0	**	*	*
RuBPCase/BSP (mg/mg)	0.41	0.49	0.53	0.55	NS	**	*
RuBPCase/Chl (mg/mg)	5.93	6.49	7.36	6.20	NS	NS	*
RuBPCase/DNA (mg/mg)	25.8	26.4	30.9	25.3	*	NS	NS
RuBPCase activity							
nmol CO_2/min·mg BSP	381	398	432	402	NS	NS	NS
μmol CO_2/hr·mg Chl	333	356	372	296	NS	NS	NS
nmol CO_2/min·mg RuBPCase	944	813	808	723	**	**	NS

[a] Ploidy.
[b] Location.
[c] *, ** denote significance at the 0.05 and 0.01 level, respectively.

to Chl, and RuBPCase to BSP in alfalfa leaves were similar to those reported in other C_3 species (12, 14, 24). Concentrations of RuBPCase and ratios of RuBPCase to DNA were similar across ploidy levels in both isogenic sets. Expressing the weight of gene product per unit DNA allowed comparisons between ploidy levels on a cellular basis. It can be inferred from the ratios of RuBPCase to DNA that doubling nuclear DNA per cell, as occurs with polyploidization, results in an approximate doubling of RuBPCase per cell. Cellular content of Chl also appeared to double with ploidy because ratios of Chl to DNA were not significantly different across ploidy levels. These results are consistent with data from leaf protoplasts isolated from the DDC 2X-4X and IC 4X-8X alfalfa populations (17, 18). Ratios of RuBPCase to DNA, chloroplast thylakoid-bound coupling factor 1 to DNA, and Chl to DNA were not significantly different between alfalfa protoplasts from different ploidy levels (17, 18). The number of chloroplasts per cell was observed to double with ploidy in the DDC 2X-4X and IC 4X-8X populations (18).

Activities of fully activated RuBPCase expressed per mg BSP or per mg Chl were not significantly different between ploidy levels of both isogenic alfalfa sets. These activities represent maximal activity of RuBPCase in leaf extracts because the enzyme was completely activated and assayed with saturating substrate concentrations. In an allopolyploid series of tall fescue (12), RuBPCase specific activities were not significantly different between ploidy levels when RuBPCase was fully activated. The earlier observation that a decaploid tall fescue genotype had a higher RuBPCase specific activity than hexaploid genotypes (22) was later attributed to incompletely activated enzyme (12). Jensen and Bahr (11) have suggested that photosynthetic CO_2 assimilation may be regulated by the amount of RuBPCase and the degree to which the enzyme is activated. Steady-state photosynthesis at irradiances of 225 to 1650 $\mu E \cdot m^{-2} \cdot s^{-1}$ is limited by activation of RuBPCase which varied from 20 to 60% of full activation (20).

Garrett (7) reported that purified RuBPCase from tetraploid ryegrass (*Lolium perenne* L.) had both a lower apparent $K_m(CO_2)$ value and a higher isoelectric point than RuBPCase isolated from comparable isogenic diploids. Rathnam and Chollet (23) found that diploid ryegrass protoplasts had both a higher apparent $K_m(CO_2)$ value and a greater rate of photorespiratory glycolate formation relative to photosynthesis than did tetraploid protoplasts. However, subsequent studies by McNeal *et al.* (16) and Rejda *et al.* (24) showed no significant differences between isogenic diploid and tetraploid ryegrass in apparent $K_m(CO_2)$ values of RuBPCase; kinetics for deactivation and activation of the CO_2/Mg^{2+}-activated and -depleted RuBPCase, respectively; and isoelectric points and molecular weights of RuBPCase subunits. With partially purified enzyme from the DDC 2X-4X and IC 4X-8X alfalfa populations, no differences were observed between ploidy levels in apparent $K_m(CO_2)$ values and substrate specificity factors (V_cK_o/V_oK_c) of RuBPCase/oxygenase (25).

CONCLUSIONS

Previous studies concerning the physiological and biochemical effects of nuclear polyploidization have often suffered from the confounding influences of inbreeding depression associated with colchicine-induced polyploids (3, 26, 29) or genetic differences between allopolyploid genotypes (13). In the present study, comparisons of ploidy effects were made between isogenic alfalfa populations with equivalent hybrid states.

Ploidy has been reported to alter mesophyll cell volume (5) and the area, shape, and weight of leaves (6, 26, 30). Therefore, the basis of expression of physiological and biochemical data becomes important for meaningful comparisons between ploidy levels. Understanding of the consequences of polyploidization is facilitated by expressing the concentrations of enzymes and other cellular constituents on a per cell or per unit DNA basis rather than on a per leaf area or per leaf weight basis. In the DDC 2X-4X and IC 4X-8X sets of alfalfa, ratios of RuBPCase to DNA and Chl to DNA were similar across ploidy levels, suggesting that cellular content of Chl and RuBPCase increases proportionately with the amount of DNA per cell.

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