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Ploidy Effects on Anatomy and Gas Exchange of Tall Fescue Leaves[1]

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ABSTRACT

A growth chamber study was designed to interpret differences in CO_2 exchange rate (CER) and leaf diffusive resistance among 4X, 6X, 8X, and 10X ploidy levels of tall fescue (*Festuca arundinacea*, Schreb). Mesophyll cell size, stomatal density, number of major and minor veins, and bundle cap size of leaf blades were evaluated. Diffusive resistance tended to decrease and CER to increase with increasing ploidy level. Mean stomatal density decreased from 43.6 per square millimeter to 30.6 per square millimeter as ploidy level increased from 4X to 8X. The 10X ploidy level exhibited the highest stomatal density, 47.2 per square millimeter. Major veins decreased from a mean of 14.2 to 10.2, and minor veins increased from a mean of 4.2 to 6.6, per leaf blade as ploidy increased from 4X to 10X. Total number of veins decreased significantly from a mean of 18.4 to 15.7 as ploidy increased from 4X to 8X.

Length and width of mesophyll cells tended to increase as ploidy increased from 4X to 8X, but then decreased again at 10X. The number of cells in the bundle cap showed no trend among ploidy levels. Estimated volume of mesophyll cells increased six times between the 4X and the 6X level while chromosome number of nuclear DNA per cell increased only 50%. However, increases in estimated cell volume were proportional to chromosome number as ploidy increased from 6X to 8X. The relationship between cell volume and chromosome number at 10X was intermediate between that at 4X and 6X or 8X.

Differences in stomatal density and diffusive resistance did not fully account for the ploidy effect on CER. Further mesophyll cell volume was positively related to CER, a factor contrary to earlier experiments.

Tall fescue (*Festuca arundinacea*, Schreb) exhibits a polyploid series that includes 28 (4X), 42 (6X), 56 (8X), and 70 (10X) somatic chromosomes. Randall *et al.* (10) reported that a 10X genotype of tall fescue displayed a CER[4] that was 49 to 91% higher than the mean of the normally cultivated 6X genotypes. Since the decaploid was found to have 30 to 100% higher specific activity of RuBP carboxylase than did the typical hexaploid genotypes, altered genetic expression of RuBP carboxylase was suggested. However, the effect of ploidy may also be related to physical processes in gas exchange. Therefore, we examined anatomical characters, leaf diffusive resistance, and a broader range of genotypes to evaluate the relationship between ploidy and CER.

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[4] Abbreviations: CER, carbon dioxide exchange rate; RuBP, ribulose-1,5-bisphosphate.

Increased ploidy level in the 2X to 8X range was associated with a decreased stomatal density in currant (*Ribes satigrum* Syme) (1), scarlet datura (*Datura stramonium* L.) (4), kohlrabi (*Brassica oleracea* L. var. *gonglylodes*) (6), triticale (X *Triticosecale*) (12), bromegrass (*Bromus inermis* Leyss) (14), and alfalfa (*Medicago sativa* s.l.L.) (13). This decrease in stomatal number was generally accompanied by an increase in stomatal size (4, 12–14, 16). Rates of photosynthesis and transpiration often closely follow changes in stomatal aperture (8, 18).

Anatomical characteristics were associated with differences in leaf rigidity of *Lolium* spp. (11) and may be important to agronomic performance. Increase in nuclear ploidy was associated with an increase in cell volume in fern gametophytes (5) and with longer and wider leaves in bromegrass (14). An anatomical characterization of polyploid tall fescue would be helpful in developing an understanding of photosynthetic efficiency and other physiological mechanisms of intact leaves that may be influenced by ploidy differences.

Our objectives were to characterize anatomically leaf blades of four ploidy levels of tall fescue, examine ploidy influence on CER and leaf diffusive resistance, and determine whether gene dosage or ploidy effects were associated with altered cell size in tall fescue.

MATERIALS AND METHODS

The four ploidy levels of tall fescue used were tetraploid (2N = 4X = 28 chromosomes), hexaploid (2N = 6X = 42), octaploid (2N = 8X = 56), and decaploid (2N = 10X = 70). Each ploidy level was represented by four genotypes, with an effort being made to select genotypes that were introduced primarily from the Atlas mountain area of the Mediterranean region (2). This meant that the ecological range among ploidy levels during evolution was narrower than that of random selections.

Plants were propagated vegetatively from clones grown in the field at the Agronomy Research Center, Columbia, MO. Ramets were transplanted into plastic pots (11.5 cm in diameter × 14.5 cm high) containing a potting mixture of two parts Mexico silt loam topsoil and one part sand. Plants were grown in the greenhouse at 25 to 35 C for 3 months. Two weeks prior to sampling, plants were placed in a growth chamber at 25/20 C (light/dark) with a 12-h photoperiod and 70% RH. Photosynthetic photon flux density (400–700 nm) of about $500\mu E \cdot m^{-2} \cdot s^{-1}$ was provided by cool-white fluorescent and incandescent lamps.

The CER at $500\mu E \cdot m^{-2} \cdot s^{-1}$ was measured with a Beckman Model 215A infrared CO_2 analyzer using an open system and leaf chamber as described by Nelson *et al.* (9). Diffusive resistance was measured with a Lambda Instruments Model LI-60 porometer.

The middle parts of recently collared leaf blades were removed and placed in FAA solution (70% [v/v] ethanol:formaldehyde: glacial acetic acid [40:5:5]) for 2 to 5 days in order to clear and preserve the tissue. Five replications (pots) of each genotype were sampled.

Flat sections were prepared by cutting 3-mm × 3-mm segments

from the cleared leaf blade tissue. The segments were then dipped in Hematoxylin Stain Solution (Harris-Lillie, Fisher Scientific Company, Fair Lawn, NJ), rinsed, and mounted in glycerol. Cross sections were prepared by placing a cleared leaf sample between two halves of elder (*Sambucus canadensis* L.) pith that had been split longitudinally. A sharp razor blade was then drawn transversely across the pith to obtain very thin leaf sections. Sections were dipped in Hematoxylin Stain Solution, rinsed, and mounted in glycerol.

Length and width of epidermal cells, number of stomata per mm^2, and number of adjacent rows of stomata were determined with a light microscope from flat sections of the upper surface at 100X. Height and width of mesophyll cells (400X), number of major and minor veins (100X), and size of structural tissue (100X) were determined on cross sections. Structural tissue referred to the bundle cap which was at the top of each veinal ridge, and its size was estimated by counting the number of schlerenchyma cells. Number of mesophyll cells along the upper leaf epidermis and between bundle caps was counted on cross sections.

RESULTS AND DISCUSSION

On a leaf weight basis, CERs of octaploid and decaploid genotypes were significantly higher ($P < 0.10$) than those for tetraploid and hexaploid genotypes, and a significant correlation ($r = 0.59$, $P < 0.05$) occurred between CER per unit leaf weight and ploidy level (Table I). Stomata on the upper surface were located on either side of the veins and were often oriented in a series of adjacent rows. Up to seven adjacent rows of stomata were observed among genotypes, but no trend was evident between this character and ploidy level. The number of adjacent rows of stomata was correlated positively ($r = 0.52$, 77 *df*, $P < 0.01$) with stomatal density. Therefore, in this case, stomatal density did not appear to be a differentially limiting factor to CER. On a leaf-area basis, CER exhibited much the same trend among ploidy levels as it did on a dry weight basis (Table I), with a correlation of 0.92 ($P < 0.01$). This supports the conclusion of Wilhelm (15), who investigated dry weight and leaf area as a basis to express CER differences among hexaploid genotypes.

Knowing mesophyll cell dimensions, mesophyll cell number serves as a measure of the relative distance between bundles in a leaf section. Crookston and Moss (3) found that C_3 species had 9 to 15 mesophyll cells between vascular bundles while C_4 species had two when counting cells directly from one bundle to the next from a paradermal leaf section. They did not report interspecific differences within the C_3 or C_4 groupings. Only the peripheral cells were counted in our study (*i.e.* those adjacent to epidermal or bulliform cells and along the upper leaf surface); thus, our data cannot be compared directly with that of Crookston and Moss (3).

Table I. *Influence of Ploidy Level on Stomatal Density on the Upper Surface, Diffusive Resistance, and CER of Tall Fescue Leaf Blades*

Data for stomatal length are from Hake (7).

Ploidy	Stomata		Diffusive Resistance		CER	
	Density	Length	Upper	Lower	g Dry wt	dm^2 Leaf area
	$No \cdot mm^{-2}$	μm	$s \cdot cm^{-1}$		$mg\ CO_2 \cdot h^{-1}$	
4X	43.6	41.8	6.7	17.7	32.7	15.9
6X	36.3	45.5	2.4	5.5	34.6	18.8
8X	30.6	45.8	2.4	8.8	46.6	22.3
10X	47.2	50.7	0.8	3.7	44.4	21.7
LSD						
0.05	8.5	3.5	3.6	4.3		
0.10					5.6	2.0

However, we found that the number of mesophyll cells between bundles averaged 22 and was not significantly different among ploidy levels (data not shown).

Stomatal density decreased with increasing ploidy from 4X to 8X (Table 1), but, in 10X genotypes, the stomatal density was the highest of the four ploidy levels. Our findings of lower stomatal density with higher ploidy level up to 8X was consistent with that of several other species (1, 6, 12–14). However, the high stomatal density of the decaploids did not follow the trend. Hake (7), working with the same genotypes, observed a trend of increased stomatal length with increased ploidy from 4X to 8X (Table I). However, the increased stomatal size was not sufficient to offset the effect of decreased stomatal density such that the total stomatal pore space per unit area decreased.

Contrary to expectation, stomatal density on the upper leaf surface was not related to high diffusive resistance (Table I). Even though the tetraploid and decaploid genotypes showed the highest stomatal densities, they had widely varying diffusive resistance. This phenomenon could possibly be explained by differences in response of stomata to environment (19), but plants were kept well watered during the experimental period, and all plants experienced the same environment. At the decaploid level, high stomatal density was accompanied by low diffusive resistance. There was a significant negative correlation between ploidy level and diffusive resistance on both the upper ($r = -0.61$, $P < 0.05$) and lower leaf surfaces ($r = 0.57$, $P < 0.05$).

Both width and length of mesophyll cells in leaves of tetraploid plants were significantly smaller than were those in hexaploid, octaploid, and decaploid plants (Table II). There was a trend of increasing mesophyll cell size as ploidy increased from 4X to 8X, just as there was a decrease in stomatal density (Table I). Since length and width of mesophyll cells were highly correlated ($r = 0.99$, $P < 0.01$), we assumed that height would be proportional, and we estimated mesophyll cell volume (Table II). Volume increased from 4X to 8X with the 10X being intermediate.

A possible implication of ploidy on CER would be that higher ploidy levels have fewer veins or less nonphotosynthetic tissue in leaves. More or less veins could also alter translocation. Veins were classified as major if there was extensive development of a collenchymatous bundle sheath. In major veins, the bundle sheath was one to three cells thick and was continuous with a small bundle cap. Minor veins were not completely surrounded by a layer of collenchyma cells, and the bundle cap was separated from the bundle sheath by one to three layers of mesophyll cells.

Major veins decreased and minor veins increased with increasing ploidy level (Table II). Total number of veins tended to decrease as ploidy level increased from 4X to 8X, but the number was intermediate at 10X. The decrease in total vein number was most likely a function of leaf width, as the number of mesophyll cells between veins remained similar among ploidy levels. Based on anatomy, the number of veins did not appear to be a major factor in CER.

In the 4X to 8X range of ploidy, the volume of mesophyll cells increased with gene dosage. The 6-fold increase in mesophyll cell volume as ploidy increased from 4X to 6X was much larger than the 50% increase in chromosome number. As ploidy increased from 6X to 8X, however, increase in cell volume was proportional to the increase in chromosome number. The decaploid genotypes exhibited an estimated mean cell volume that was lower than both the hexaploid and octaploid genotypes and, as a result, decaploid cells had less volume per chromosome. Therefore, a range in chromosome number per unit leaf area is possible and may be a factor related to genetic control of physiological processes.

The fact that both mesophyll cell area and CER increased from 4X to 8X is contrary to data of Wilson and Cooper (17), who found a negative correlation between mesophyll cell size and photosynthetic rate within a ploidy level of perennial ryegrass

Table II. *Influence of Ploidy Level on Mean Values of Some Anatomical Characteristics of Tall Fescue Leaf Blades*

Ploidy	Veins			Mesophyll Cell			Structural Tissue	
	Major	Minor	Total	Length	Width	Estimated Volume[a]	Height	Width
		No.			*μm*		*No. of cells*	
4X	14.2	4.2	18.4	18.8	10.9	1.0	6.05	3.10
6X	13.9	3.4	17.4	34.3	19.8	6.0	4.75	2.75
8X	10.7	5.0	15.7	38.1	20.5	7.2	6.10	2.45
10X	10.2	6.6	16.8	31.8	18.2	4.7	6.28	2.94
LSD								
0.05	1.7	1.0	0.8	7.5	7.0		0.86	0.56

[a] Volume index assuming 3rd dimension was proportional to width and 4X was equal to 1.0.

(*Lolium perenne* L.). However, the polyploid series employed in our study was not homogeneous; *i.e.* the four ploidy levels were evolved from different parental lines, and gene action would not be expected to be strictly additive. Since the additive nature of the genetic material may vary among ploidy levels, it is difficult to assess the relative contributions of genetic and physical factors with regard to photosynthetic rate.

The 10X genotypes exhibited an atypically high stomatal density and intermediate mesophyll cell volume which may be related to other characteristics of decaploid tall fescue genotypes such as high CER and high RuBP carboxylase activity (10). The fact that diffusive resistance appeared to decrease with increasing ploidy level indicated that the diffusive resistance was a result of many factors, not just stomatal density. Anatomical features appear to have a moderating influence, but are probably not the major factor associated with the expression of higher CER as ploidy level is increased.

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