Photosynthesis in Fescue[1]

III. RATES OF ELECTRON TRANSPORT IN A POLYPLOID SERIES OF TALL FESCUE PLANTS

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ABSTRACT

Photosystem I electron transport activity has been found to be considerably higher in a decaploid tall fescue (*Festuca arundinacea* Schreb.) genotype as compared to a common hexaploid genotype. The decaploid genotype also displayed a higher photosystem whole chain (Photosystem II plus Photosystem I uncoupled) activity, suggesting a connection between polyploidy and increased electron transport activity. However, when a polyploid series of tall fescue, ranging from diploid to decaploid with several different genetic isolates at each ploidy level, was examined in natural growth conditions, no effect of increasing genome content on electron transport and photophosphorylation was found. These results suggest that a gene component of one of the genomes involved may be responsible for the increased activity rather than simply the total chromosome content.

Stebbins (23) suggests that the most widespread process affecting the evolution of higher plants is the multiplication of entire sets of nuclear chromosomes, or the phenomenon of polyploidy. The most immediate effect of polyploidy is often seen in changes of lead morphology which are visible in increased cell and organ size. While the morphological effects are most obvious, other physiological differences occur as chromosome level is changed. Decreased photosynthesis (1, 6), decreased growth rate (7), and reduced reproductive ability (23) are only a few of the negative occurrences that accompany polyploidy. In most instances, the simple doubling of chromosome sets is not advantageous to the evolution of higher plants, but the doubling must be accompanied by hybridization or introduction of new chromosome sets in order that the new genotype become successful (23). It is probably this combination of events, polyploidy and hybridization, that had enabled tall fescue, *Festuca arundinacea* Schreb., to become a successful hexaploid (2n = 42).

The suggested correlation between increased chromosome number and photosynthetic activity has been a difficult phenomenon to prove. Dunbier *et al.* (8), using diploid and tetraploid alfalfa, provided good evidence that polyploidy positively affected dry matter yield. Setter *et al.* (22), using diploid and tetraploid colchicine-treated alfalfa, found no effect of polyploidy on CO_2 exchange rate and transpiration when data were expressed per unit leaf area. Randall *et al.* (18) has suggested increased CO_2 exchange

rate and RuBP[2] carboxylase activity to accompany a change from hexaploid to decaploid in genotypes of tall fescue. Recently, Ratham and Chollet (19) confirmed work by Garrett (9) with diploid and tetraploid ryegrass genotypes by showing a correlation between decreased photorespiration (and therefore increased CO_2 exchange rate) and polyploidy. Only in the latter studies, are leaf morphology changes accounted for in the biochemical analysis, giving strong evidence to the direct influence of polyploidy on the chloroplast. When only two genotypes are examined, as in all the studies above, the difficulty of suggesting a change due only to polyploidy becomes more acute.

In our previous work (14), we have shown that isolated chloroplasts from hexaploid tall fescue can exhibit high rates of chloroplast electron transport and phosphorylation. Here, we compare the commonly cultivated hexaploid to a polyploid series of tall fescue with nuclear chromosome number of 14 in the diploid through chromosome number 70 in the decaploid. We used isolated, functional chloroplasts to avoid effects of any changes in leaf morphology that might have occurred. Part of this same series of genotypes has been examined for RuBP carboxylase activity and CO_2 exchange rate activity (13, 18). Convincing evidence has been presented showing a positive functional relationship between increasing chromosome number and increased activity.

Initially, we compared the high performing decaploid, I-16-2 (11), to the hexaploid KY-31 (14), and we found PSI electron transport activity to be consistently higher in the decaploid, while PSII + I electron transport was only slightly higher in the decaploid. The increased activity suggests that increasing chromosome number might be related to increasing photosynthetic activity (18). Yet, when 24 genotypes in a polyploid series were examined, no correlation between polyploidy and photosynthetic activity was found. Our conclusion was that the decaploid genotype contains the gene(s) responsible for the increased PSI electron transport activity, which may be transferable within this genus.

MATERIALS AND METHODS

Plant Material. Clones of selected tall fescue genotypes were vegetatively propagated from tillers and placed into 4-inch pots for growth chamber and greenhouse studies. Plant material grown in the growth chamber was kept at a 14-h (400 μE m^{-2} s^{-1}), 25-C day and a 10-h, 20-C night. Nine pots representing three repetitions for each genotype were planted in a selected plot on the southeast corner of the University of Missouri, Columbia, campus in late April, 1980. Analysis was done on this plot for field studies in early October, 1980. Tissue harvested for chloroplast isolation

[2] Abbreviations: RuBP, ribulose 1,5-bisphosphate; TMQH$_2$, tetramethyl-*p*-hydroquinone; MV, methyl viologen; DAD, diaminodurene; TMQ, tetramethyl-*p*-benzoquinone; PD$_{ox}$, oxidized-*o*-phenylenediamine; K_{app}, K apparent; DBMIB, dibromothymoquinone; SOD, superoxide dismutase; ASC, sodium ascorbate.

was cut from young, fully expanded leaves as previously described (14). Chl content and Chl a to Chl b ratios were determined by extraction in 80% acetone, and absorption was measured on a Perkin-Elmer 552 spectrophotometer, as described by Arnon (2).

Electron Transport and Phosphorylation Measurements. All of the assays were performed in a 5.8-ml water-jacketed (25 C) reaction chamber, with the exception of the TMQH$_2$-to-MV assay, which was done in a 2.9-ml reaction chamber. Oxygen changes were measured by a Clarke-type O$_2$ electrode (Yellow Springs Instrument) with conditions as previously described (14). Actinic light (6,000 μE m^{-2} s^{-1}) was provided by a projection lamp equipped with a heat trap. Experiments using TMQH$_2$ as the electron donor (9, 21) were done with a red cut-off filter (575 nm) that allowed greater than 40% of the 650- to 700-nm red light to pass as actinic light. Phosphorylation was measured as [^{32}P]ATP formation (14, 20).

Chemicals. General chemicals were purchased from Sigma and Mallinckrodt Chemical Co., with the exception of DAD (Eastman). TMQ, DAD, and PD$_{ox}$ were recrystallized after acquisition and dissolved prior to use. TMQ was reduced to TMQH$_2$, as described by Izawa and Pan (12). [^{32}P]Pi was purchased from New England Nuclear.

RESULTS

Table I shows a comparison of the light reactions of the common hexaploid to the high-performing decaploid I-16-2, both grown in the growth chamber conditions. Except for cyclic phosphorylation, the decaploid shows higher rates of electron transport and phosphorylation than does the hexaploid. In all reactions, the decaploid had higher electron flow rates as well as higher ATP synthesis. While the P:2e in PSII of the decaploid is greater than in that of the hexaploid, the P:2e was lower in the PSI and PSII + I reaction as a result of a large increase in electron flow. These data suggest that the decaploid is less efficient photosynthetically than is the hexaploid.

Both genotypes were examined more closely for PSII activity (Fig. 1) and to see the significance of the electron flow difference seen in Table I. Both genotypes were found to have near identical rates at low and high light intensities. Response to varying light intensities with neutral filters and the near total inhibition of electron flow by DCMU (>95%) suggests that the two genotypes have near identical PSII activity.

However, when PSI activity was measured and compared at varying light intensities, a large difference in the electron transport rate could be detected (Fig. 2). The rate difference was detectable at low intensities as well as at high intensities. The decaploid maximum rate of DAD-to-MV electron flow was significantly greater than the hexaploid maximum flow. When the data were replotted as a Woolf-Augustinsson-Hofstee plot (21), not only was the maximum rate (V_{max}) difference more visible but the K_{app} was also found to be different (Fig. 2B). A second type of the PSI difference is shown in Figure 3, where the artificial electron donor

concentration was varied. Again, at nearly all but very low concentrations, the electron transport for the decaploid was higher than for the hexaploid.

A second donor to PSI was used to assay PSI activity, but this donor (TMQH$_2$) was DBMIB-sensitive, providing electrons on the PSII side of the plastoquinone pool (12, 25). The electron transport in the decaploid was greater than in the hexaploid (Table II), but the difference was not as great as that of the DAD donor in a DBMIB-insensitive assay. Electron transport was 13% higher, and ATP synthesis was 30% higher, for the decaploid, resulting in a P: 2e of 0.79 for the decaploid and 0.59 for the hexaploid.

After examination of a limited number of the growth-chamber grown genotypes, a complete study of the light reactions in isolated chloroplasts from an extended series of greenhouse- and field-grown polyploid genotype clones was performed and evaluated (Figs. 4–6). Figure 4 shows PSII activity of all genotypes, diploid (2n = 14) through decaploid (2n = 70). Assays performed on greenhouse-grown clones showed slightly higher rates than did those performed on field-grown clones, but there appears to be no trend or indication of increasing or decreasing rates of electron transport as chromosome number changes.

Figure 5 shows PSI activity of all genotypes in the ploidy series, grown under both conditions. While a few decaploids, such as I-16-2 (m) and PI-283-283-5 (n), have very high rates of electron transport, there is no increasing trend in activity as chromosome number increases. Poorly performing genotypes include the octaploids. As with PSII, activity in greenhouse-grown clones was higher than that in field-grown clones.

When overall uncoupled PSII + I electron transport rates were measured (Fig. 6), a trend similar to that of PSI was found. Several tetraploids performed equal to the decaploids, with the octaploids displaying the poorest rate of electron transport activity. Yet, there is no correlation between increasing chromosome number and increasing or decreasing photosynthetic activity.

When Chl concentrations were determined at the time of chloroplast isolation, Chl a to Chl b ratios were obtained for all genotypes. Results of the greenhouse-grown clones exhibited a distribution of ratios with the minimum of 2.08 ± 0.11 SD in the diploid and maximum of 2.55 ± 0.16 in the decaploid. Comparatively, all field-grown clones had higher Chl a to Chl b ratios, ranging from 2.73 ± 0.40 in the diploid and 2.86 ± 0.09 in the hexaploid to 3.07 ± 0.10 in the decaploid and in the tetraploid F. mairei. There was no pattern of changing ratios with increasing ploidy number.

DISCUSSION

The use of polyploidy was once thought to be a simple research tool that would revolutionize the plant breeding industry (24). Unfortunately, this genetic tool has never lived up to these expectations. Nevertheless, polyploidy seems to occur in higher plants, such as grasses, in nature as the rule rather than as the exception (23). To date, there is no direct relationship between nuclear

Table I. *Phosphorylation and Electron Transport of Chloroplasts from Hexaploid KY-31 and Decaploid I-16-2 Genotypes*

Genotypes	Cyclic[a] Phospho-rylation	PDII[b]			PSI[c]			PSII + I[d]		
	μmol Pi	μeq e$^-$	μmol Pi	P:2e	μeq e$^-$	μmol Pi	P:2e	μeq e$^-$	μmol Pi	P:2e
6x Hexaploid (KY-31)	480	1,300	319	0.50	3,180	568	0.42	228	152	1.33
10x Decaploid (I-16-2)	483	1,600	445	0.55	4,044	726	0.35	432	236	1.10

[a] Pyocyanin, 30 μM.

[b] H$_2$O → PD$_{ox}$.

[c] DAD/ASC → MV; +8.6 μM DCMU, +300 units per ml SOD.
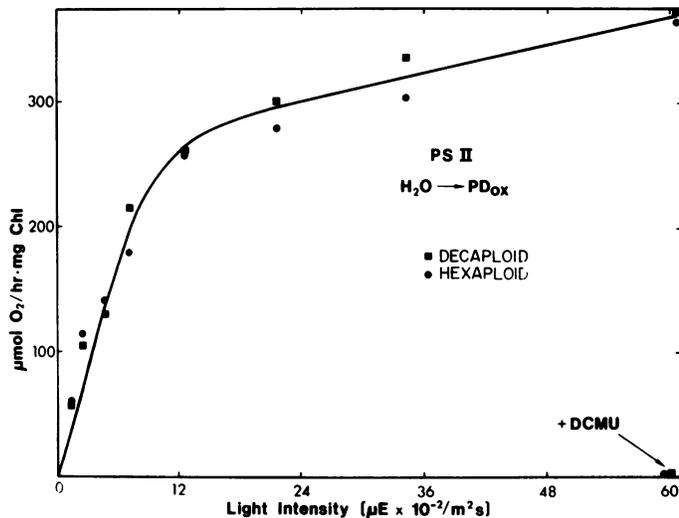
[d] H$_2$O → MW.

FIG. 1. PSII electron transport of isolated chloroplasts from the common hexaploid KY-31 and decaploid I-16-2 genotypes assayed under varying light intensities. Assay conditions include 40 mM Tricine (pH 8.0), 4.0 mM $MgCl_2$, 60 mM NaCl, 20 to 25 μg Chl of chloroplast suspension, 400 μM PD_{ox}, and 2.0 mM potassium ferricyanide.
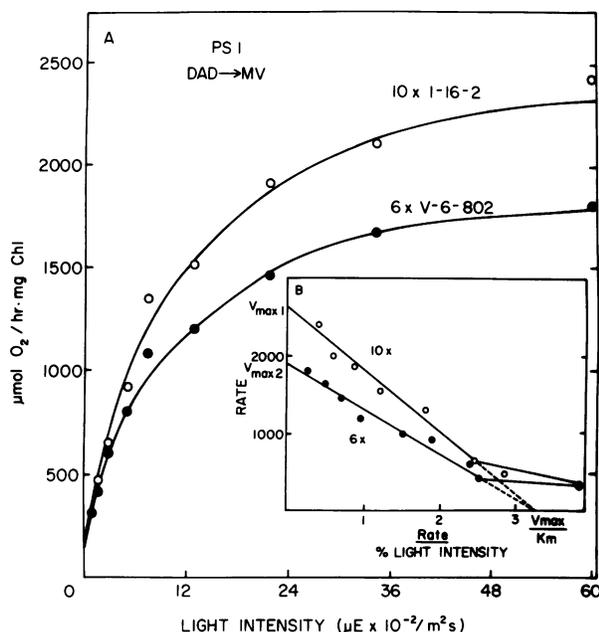

FIG. 2. A, PSI electron transport of isolated chloroplasts from hexaploid KY-31 and decaploid I-16-2 genotypes. Specific reaction conditions as in Figure 1 legend, except that the donor was 0.5 mM DAD and 2.0 mM ASC, and the acceptor was 100 μM MV. B, Data were replotted as a Woolf-Augustinsson-Hofstee plot.

chromosome content or quantity and chloroplast function. Genetic studies have led researchers to nuclear mutations that seem directly to affect chloroplast-coded proteins (16), but the direct effect of doubling or tripling total nuclear content on biochemical functions in the light reactions of photosynthesis in the chloroplast is still unclear. The increasing gene content can positively affect plant functions, specifically photosynthetic capacity (1); however, the process is usually not through exclusive self-duplication of genomes (autopolyploidy) but rather through hybridization and polyploidy (alloautopolyploidy).

Tall fescue, *F. arundinacea* Schreb., section Bovinae, has been shown to have a polyploidy series 2n = 28, 42, 56, and 70 (3), with
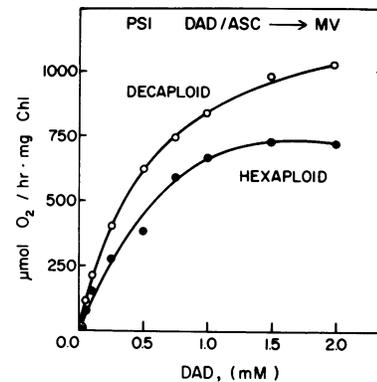

FIG. 3. PSI electron transport of isolated chloroplasts from hexaploid KY-31 and decaploid I-16-2 genotypes. See Figure 1 for reaction conditions. The donor was DAD and 2.0 mM ASC in the presence of 8.6 μM DCMU and 250 units of SOD per ml.

the diploid (2n = 14) known in the Bovinae section as *F. pratensis* (15). Because this series has successfully established itself to temperate regions in Europe, North Africa (3), and North America (4), it is in our interest to explore the biochemical adaptations in photosynthesis that have occurred as the nuclear genome chromatin content has increased. These studies include the diploid, *F. pratensis*, a plant that has contributed to the evolution of tall fescue (15); the tetraploid, *F. mairei*, thought to be the hybrid link between the sections Bovinae and Scariosae (3); and the tetraploid, *F. arundinacea* var. glaucesens, and hexaploid, octaploid, and decaploid genotypes of *F. arundinacea*. This line of polyploidy has been assembled through hybridization but has been found to have a structural genetic similarity as evidence of a high degree of autosyndesis; *i.e.* the pairing in a polyploid of chromosomes which are derived from the same parent (5, 15). In all but the diploid, *F. pratensis*, there is species hybridization with little autoploidy, probably contributing to the success of almost all genotypes of fescue.

A starting point of photosynthetic analysis of the tall fescue polyploid series came from Randall *et al.* (18), who found that a decaploid genotype of tall fescue, I-16-2, displayed high CO_2 exchange rate and RuBP carboxylase activity when compared to a hexaploid genotype. The suggestion from these findings was of a potential link between increasing photosynthesis and increasing nuclear genome content. Later, findings from the same laboratory (13) stated that plant clones from the same fescue polyploidy series, tetraploid through decaploid, had increasing net photosynthesis when based on leaf weight. Also, a suggestion was made (13) that ploidy selectively increased the concentration of RuBP carboxylase in the total soluble protein fraction.

We began our study by examining electron transport and photophosphorylation rates in the high-performing decaploid genotype, I-16-2 (11, 18), in comparison to a common well-adapted hexaploid cultivar, KY-31 (4, 14). Early analysis indicated that the decaploid had higher rates in all systems except cyclic phosphorylation. When PSII and PSI electron transport rates were examined under optimal phosphorylating conditions, these rates were nearly 20% higher in the decaploid, and PSII + I rates were over 40% higher in the decaploid. Explanation for the similarity of the decaploid and the hexaploid in the rates of ATP synthesis during cyclic electron flow (using the artificial mediator, pyocyanin) could be based on the short shuttle model presented by Hauska *et al.* (10). Because the cycle includes P700, PSI, and the primary electron acceptor of PSI, differences between the decaploid and the hexaploid might be lost.

Both PSII and PSI were examined more closely, varying the light intensity and donor/acceptor concentration. PSII electron transport showed no differences between the two genotypes as

Table II. *PSI Phosphorylation and Electron Transport of Chloroplasts from Hexaploid KY-31 and Decaploid I-16-2 Genotypes*

Reaction mixture as indicated in Table I, except volume was 2.8 ml, and PSI donor used was 0.5 mM $TMQH_2$ without ASC but with 8.6 μM DCMU and 300 units per ml of SOD.

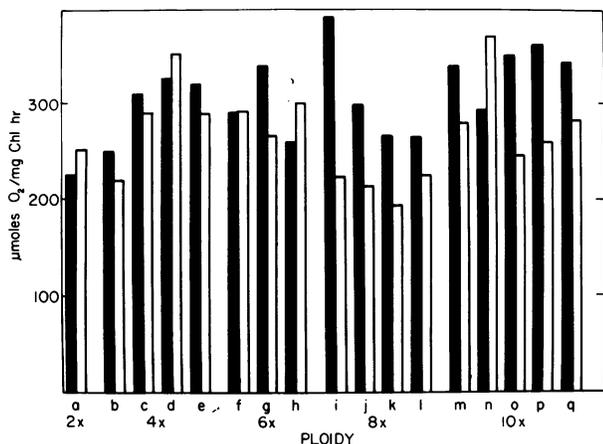| Genotype	Trial No.	Electron Transport	ATP Synthesis	P:2e
		$\mu mol\ O_2/mg$ $Chl \cdot h$	$\mu mol\ Pi/mg\ Ch \cdot h$	ratio
6x KY-31	n = 12	531 ± 66[a]	292 ± 24[a]	0.59 ± 0.03[a]
10x I-16-2	n = 11	614 ± 90[a]	427 ± 38[a]	0.79 ± 0.25[a]

[a] SE.

FIG. 4. PSII electron transport of isolated chloroplasts from 24 genotypes of fescue grown under greenhouse (■) and field (□) conditions. See Figure 1 for specific assay conditions. Diploid genotype (a) was three clones of *F. pratensis*. Tetraploid genotypes were: (b), Bn 354-5; (C), Bn 354-2; (d), Bn 574-2; and (e), *F. mairei*, three different clones. Hexaploid genotypes were: (f), I-18-3; (g), I-86-3; and (h), Bn 257-5. Octaploid genotypes were: (i), PI-283-283-6; (j), Bn 867-4; (k), Bn 275-3; and (l), I-20-15. Decaploid genotypes were: (m), I-16-2; (n), PI-283-283-5; (o), Bn 275-3; (p), PI-316-246-1; and (q), Bn 272-1.
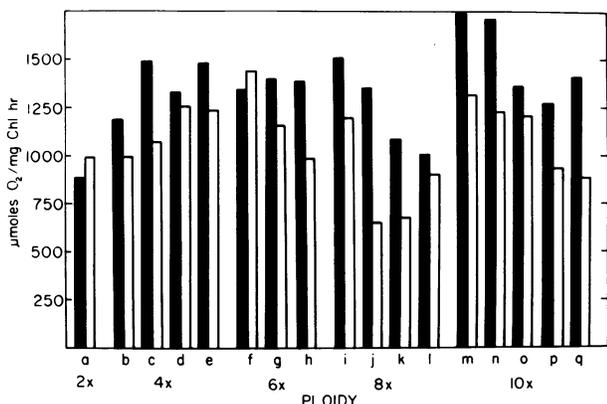

FIG. 5. PSI electron transport of isolated chloroplasts from 24 genotypes of fescue grown under greenhouse (■) and field (□) conditions. See Figure 3 for specific reaction conditions and Figure 4 for definition of individual genotypes.
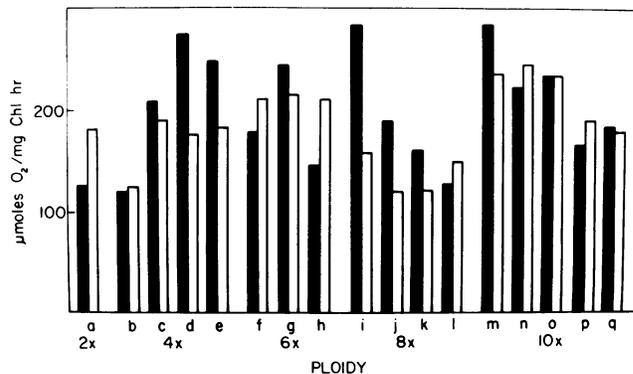

FIG. 6. PSII + I uncoupled electron transport of isolated chloroplasts from 24 genotypes of fescue grown under greenhouse (■) and field (□) conditions. Uncoupler used was 10 mM methylamine, and terminal electron acceptor was 100 μM MV with 45 to 50 μg Chl per reaction.

light intensity was changed, unlike the rates for the photophosphorylating conditions presented in Table I. However, the PSI measurements under varying light intensities and varying donor concentrations showed consistently higher rates in the decaploid. While absolute rates are not comparable to the other assays because conditions were not optimized for phosphorylation and SOD was omitted from the reaction mixture, the higher electron

transport rates in the decaploid were consistent over all ranges of light intensities. The Woolf-Augustinsson-Hofstee plot of light intensity *versus* electron transport rates (Fig. 2B) shows that, not only was the maximum rate (V_{max}) of the decaploid greater, but the K_{app} was different for the genotypes. Because of the value for K_{app} is the light intensity that yields one-half V_{max} rates, either the light harvesting complex size or the transfer of light energy to the PSI reaction center or both are different in the two genotypes. Titration of electron transport rates with increasing concentrations of the artificial electron donor DAD also showed consistently higher rates in the decaploid. This consistent difference suggests that the decaploid has a higher potential rate for electron flow to P700 (17).

When $TMQH_2$ was used as a PSI electron donor, which involved electron transport through the plastoquinone component (12, 25), the rate difference between the two genotypes was still evident but decreased. Yet, the efficiency (P:2e) for the decaploid was consistently higher than that for the hexaploid. Initial conclusions were that the high PSI activity and high RuPB carboxylase activity (18) may contribute to the high net photosynthesis in the decaploid I-16-2.

However, when the hypothesis that increasing nuclear genome content increased electron transport activity was tested, results indicated that only certain specific genotypes were high performing. This could not be attributed to ploidy levels in general. Examination first was performed upon greenhouse-grown genotype clones of fescue, including the diploid, *F. pratensis*, and tetraploids, *F. mairei* and *F. arundinacea* var. glaucesens. No clear correlation was established, so the same clones were planted under field conditions and grown for six months before the electron transport rates were measured. Activity compared to greenhouse-grown clones was generally lower, probably due to a very hot 1980 Missouri summer. More importantly, no conclusive effect of polyploidy on electron transport rates could be established. Certain

decaploid genotypes, such as I-16-2, displayed high electron transport rates in PSI, but similar rates were found in tetraploid and hexaploid genotypes of fescue. Our conclusions were that, while certain species can display high PSI electron transport rates (probably involving the P700 reaction center), there were no general ploidy effects on the light reactions in isolated chloroplasts.

The significance of the results may not lie in the polyploidy effect *per se* but in the application of the transferable, controlling gene component(s). If a specific combination or set(s) of genes enables the I-16-2 genotypes to display the increased PSI electron transport and phosphorylation rates, it should be transferable to other levels of ploidy. The necessary crosses to test this hypothesis have been made and are being tested.

LITERATURE CITED

1. ABKULLAEVA KA 1977 Polyploidy and the genome control of chloroplast number and function. Proceedings of the 4th International Congress on Photosynthesis, p 1
2. ARNON DI 1949 Copper enzyme in isolated chloroplasts polyphenol oxidase in *Beta vulgaris.* Plant Physiol 24: 1–15
3. BORRILL M 1972 Studies in *Festuca.* III. The contributions of *F. scariosa* to the evolution of polyploids in sections of Bovinae and Scariosae. New Phytol 71: 523–532
4. BURNS JC, DS CHAMBLEE 1979 Adaptations. *In* RC Buckner, LP Bash, eds, Tall Fescue, Vol 20, Chap 2. American Society of Agronomy, Madison, pp 9–31
5. CHANDRASEKHARAN P, H THOMAS 1971 Studies in *Festuca.* 6. Chromosome relationship between Bovinae and Scariosae. Z Pflanzenzuecht 66: 76–86
6. CURKROVA V, N AURATOVSAKOVA 1968 Photosynthetic activity, chlorophyll content and stomata characteristics in diploid and polyploid types of *Datura stramonium* L. Photosynthetica 2: 227–237
7. DEMAGGIO AC, RH WETMORE, JE HANNAFORD, DA STETLER, V RAGHAAVAN 1971 Ferns as a model system for studying polyploidy and gene dosage effects. Bioscience 21: 313–316
8. DUNBIER MW, DL ESKEW, ET BINGHAM, LE SCHRADER 1975 Performance of genetically comparable diploid and tetraploid alfalfa: agronomic and physio-logical parameters. Crop Sci 15: 211–214
9. GARRETT MK 1978 Control of photorespiration at RuBP carboxylase/oxygenase level in ryegrass cultivars. Nature (Lond) 274: 913–915
10. HAUSKA G, S REIMER, A TREHST 1974 Native and artificial energy conserving sites in cyclic photophosphorylation systems. Biochim Biophys Acta 357: 1–13
11. HORST GL 1973 Physiological and growth responses of tall fescue (*Festuca arundinacea* Schreb.) genotypes to drought and irrigation. PhD thesis. University of Missouri, Columbia
12. IZAWA D, RL PAN 1978 Photosystem I electron transport and phosphorylation supported by electron donation to the phastoquinone region. Biochem Biophys Res Commun 83: 1171–1177
13. JOSEPH MC, DD RANDALL, CJ NELSON 1980 Net photosynthesis rates and RuBP carboxylase of polyploid tall fescue, *Festuca arundinacea* Schreb. Plant Physiol 65: S-33
14. KRUEGER RW, D MILES 1981 Photosynthesis in tall fescue. I. High rates of electron transport and phosphorylation in chloroplasts of hexaploid plants. Plant Physiol 67: 763–767
15. MALIK CP, PT THOMAS 1966 Karyotypic studies in some *Lolium* and *Festuca* species. Caryologia 19:167–196
16. MILES CD, JP MARKWELL, JP THORNBER 1979 Effect of nuclear mutation in maize on photosynthetic activity and content of chlorophyll-protein complexes. Plant Physiol 64: 690–694
17. ORT DR 1975 Quantitative relationship between photosystem I electron transport and ATP formation. Arch Biochem Biophys 166: 629–638
18. RANDALL DD, CJ NELSON, KH ASAY 1977 Ribulose bisphosphate carboxylase. Altered genetic expression in tall fescue. Plant Physiol 59: 38–41
19. RATHAM CKM, R CHOLLET 1980 Photosynthetic and photorespiration metabolism in mesophyll protoplasts and chloroplasts isolated from isogenic diploid and tetraploid cultivars of ryegrass (*Lolium perenne* L.) Plant Physiol 65: 489–494
20. SAHA S, NE GOOD 1970 Products of the photophosphorylation reactions. J Biol Chem 245: 5017–5021
21. SEGAL IH 1975 Rapid equilibrium partial and mixed type inhibitions. *In* IH Segal, ed, Enzyme Kinetics, Chap 4. John Wiley & Sons, New York, pp 210–214
22. SETTER TL, LE SCHRADER, ET BINGHAM 1978 Carbon dioxide exchange rates, transpiration and leaf characters in genetically equivalent ploidy levels of alfalfa. Crop Sci 18: 327–332
23. STEBBINS GL 1971 Chromosomal Evolution in Higher Plants. Addison-Wesley, Reading, Massachusetts, pp 125–157
24. STEBBINS GL 1979 Polyploidy in plants: unsolved problems and prospects. *In* WH Lewis, ed, Polyploidy. Plenum, New York, pp 495–520
25. WHITE CC, RK CHAIN, R MALKIN 1978 Duroquinol as an electron donor for chloroplast electron transfer reactions. Biochim Biophys Acta 502: 127–137