ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. 55, nr 1: 35-43
1986

Oxygen and radiation effects on CO_2 exchange in light and in darkness of decaploid and hexaploid tall fescue (*Festuca arundinacea* Schreb.)

JERZY W. POSKUTA*, CURTIS J. NELSON

Department of Agronomy, University of Missouri, Columbia Mo. 65211, USA

(Received: May 15, 1985. Accepted: June 24, 1985)

Abstract

Rates of apparent photosynthesis (APS), photorespiration (PR), CO_2 compensation (Γ) and dark respiration (DR) were determined on attached shoots of decaploid (70 chromosomes) and a hexaploid (42 chromosomes) genotype of tall fescue (*Festuca arundinacea* Schreb.) using an infra red CO_2 analyzer arranged in a closed circuit system. Plants were grown at a photon flux density 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (400-700 nm) and at 25°C. Measurements were made at 25°C in O_2 concentrations of 1, 21 and 100% and at irradiance of 500 or 1800 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The decaploid exhibited rates of APS that was from 26 to 46% higher in 1 and 21% O_2 but not in 100% O_2, than those of hexaploid. Rates of PR were positively related to rates of APS. Values of Γ were very similar for both genotypes, they were little affected by irradiance, and were a linear function of O_2 concentration. The percentages of PR in true photosynthesis (TPS = APS + PR) were also similar for the two genotypes, and were a linear function of O_2 concentration. Alternatively, rates of DR were by 16-26% higher in the hexaploid than decaploid genotype, and were little affected by O_2 concentration or by previous rates of APS.

Key words: Festuca arundinacea Schreb., photosynthesis, photorespiration, dark respiration, CO_2 compensation point

INTRODUCTION

It has been reported that a decaploid tall fescue had increased activity of RuBP carboxylase and higher concentration of this enzyme in leaf extracts than did a hexaploid (Randall et al. 1977, Byrne et al. 1981,

* On sabbatical leave from University of Warsaw, Poland. Permanent address: Department of Plant Physiology II, University of Warsaw, Krakowskie Przedmieście 26/28, 00-927/1 Warszawa, Poland.

Joseph et al. 1981). Recently Wong et al. (1983) reported a two-fold higher rate of apparent photosynthesis at saturating irradiance in a decaploid as compared to hexaploid genotype of tall fescue, although both had the same C-3 type plants photosynthetic intermediates. Results of Perry et al. (1983) show that fruiting genotypes of cotton as compared to photoperiodic--sensitive nonfruiting ones exhibited about 40% higher rate of APS, and simultaneously about 30% lower rate of PR. Average ratios of APS/PR were 3.7 and 4.7 and for TPS/PR were 1.6 and 2.6 for genotypes respectively. The authors concluded that kinetic properties of RuBP carboxylase/oxygenase were altered in nonfruiting genotypes, and that the possibility of genetically reducing photorespiration exists. Rinehardt et al. (1983) also reported genetic variability in kinetic properties of RuBP carboxylase/oxygensase in barley populations. McCashin and Canvin (1979) however, observed that although mutants of barley exhibited large variation in rates of APS, the existance of a direct correlation between APS and PR contradicted the idea that low rate of APS can be associated with high rate of PR. These authors also showed a linear and negative association between CO_2 compensation concentration (Γ) and APS. Meyers et al. (1982, 1983) found similar kinetic properties of RuBP carboxylase/oxygenase in isogenic diploid and tetraploid alfalfa populations. Similar results were obtained with diploid and tetraploid cultivars of ryegrass (McNeil et al. 1981, Rejda et al. 1981). Recently it has been established (Jordan and Ogren 1981, Somerville and Somerville 1983, Ogren 1984) that the relative ratios of RuBP carboxylase/oxygenase activities are a linear function of the ratios of CO_2 to O_2 concentrations. As pointed out by Ogren and Hunt (1978) any modifications in RuBP carboxylase *in vivo* must be reflected in CO_2 exchange rates of intact leaves. Further, those species with reduced PR (Brown and Brown 1975, Keck and Ogren 1976) also exhibit reduced values of Γ. An interesting consideration in studies of CO_2 exchange is the dependence of dark respiration on previous photosynthesis. Recent reports (Azcon-Bieto and Osmond 1983, Azcon-Bieto et al. 1983) indicate a species diversity for this process. These authors observed that rates of DR of wheat and spinach leaves depended largely on content of free sugars in leaves and on previous photosynthesis. Pea leaves, however, contained a high concentration of free sugars and exhibited a high rate of DR after night period which was independent from photosynthesis. The DR of leaves of tall fescue, which also contain a high level total non--structural carbohydrates, was maintained at high rate for more than 48 hours of darkness (Moser et al. 1983) This brief review of recent data indicates a controversy concerning the possibility of genetic alteration of the ratio of photosynthesis to photorespiration, and also the dependence of DR on previous APS.

We hypothesized that if of part of the reason for the higher APS of a decaploid as compared to hexaploid genotype of tall fescue was due to an altered ratio of RuBP carboxylase to RuBP oxygenase it must be reflected in altered ratios of photosynthesis to photorespiration and in altered values of Γ. The objective of our study was to determine CO_2 compensation points (Γ) and rates of APS, PR and DR of shoot tissue as influenced by oxygen concentration and irradiation in decaploid and hexaploid tall fescue genotypes. The genotypes were known to differ markedly in APS (Randall et al. 1977).

MATERIAL AND METHODS

Clones of *Festuca arundinacea* Schreb. I-16-2 (decaploid, 10X = 70 chromosomes) and V6-802 (hexaploid, 6X = 42 chromosomes) were vegetatively propagated into 10 cm diameter by 14 cm deep plastic pots containing perlite. Initially, the vegetative plants consisted a stem base with whorl of sheets that was about 6 cm long, some intact roots and young tillers. Plants were watered frequently with distilled water and twice weekly with 50 cm^3 of complete nutrient solution (Epstein 1972). They were grown at 20 C, 80% RH, and 14 h photoperiod with photosynthetic photon flux density of 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at canopy height. Plants were carefully removed from perlite when the leaves had extended 12 to 20 cm at the end of 2 weeks. Roots of the intact plants were washed with tap water and placed in a test tube filled with water. A split rubber stopper with one hole was placed around the stem base of the shoot then used to isolate the roots from shoots by sealing the roots within the test tube containing water. The intact plant, including the test tube containing roots, was placed in a plexiglass photosynthesis chamber that was surrounded by a jacket containing a 3 cm thickness of circulating water. The chamber was part of a closed circuit system of an infra-red CO_2 analyzer (Beckman 865), a flow meter, an ice trap to condense water vapour, and a pump. The system was filled with air or the appropriate O_2 mixture from a tank checked with O_2 electrode, both being humidified to about 70% of relative humidity (RH). The volume of the system was 506 cm^3 and flow rate was 1000 $cm^3 \cdot min^{-1}$.

Gas exchange measurements were made at 500 to 1800 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux density (PPFD) and constant air temperature inside the photosynthesis chamber 25 C. The gaseous atmosphere was 1, 21 or 100% O_2 and initial CO_2 concentration of about 400 $mm^3 \cdot dm^{-3}$ with the remainder being nitrogen. Shoots were illuminated with a General Electric 600 W quartzline DYH projection bulb, and PPFD (400-700 nm) was measured using LI-COR model 170 quantum/sensor photometr. Fol-

lowing CO_2 exchange measurements the area of the leaves was determined using LI-COR model 360 area meter. Rates of APS were measured as CO_2 decreased from 320 to 280 $mm^3 \cdot dm^{-3}$, then plants were allowed to continue CO_2 uptake until Γ was reached. Depending on the rates of APS, Γ was reached within 6-9 minutes. Light was turned off and DR measured between CO_2 concentrations of 280 and 320 $mm^3 \cdot dm^{-3}$ that occured after 7-10 minutes of darkness. The total duration of the light-dark cycle was less than 30 minutes. Following 30 min of adaptive illumination, the measurement procedure was repeated three or four times. The PR was calculated as $PR = CE \times \Gamma$, where CE (carboxylation efficency) equals APS (CO_2 conc. $- \Gamma$) as described by Tregunna et al. (1966). Two separate plants were measured from each of two separate experiments, all plants within a treatments giving similar results. The data were averaged and standard errors calculated.

RESULTS AND DISCUSSION

Both the decaploid and the hexaploid genotype of tall fescue had the highest rates of APS in 1% O_2 at both PPFDs (Fig. 1). The rates were considerably decreased in 21% O_2 and were lowest in 100% O_2. The largest genotypic difference occured at 1% O_2. The decaploid exhibited 26 to 46% higher rates of APS than did the hexaploid in 1 and 21% O_2 but not in 100% O_2 where rates were similar. These data are consistent with

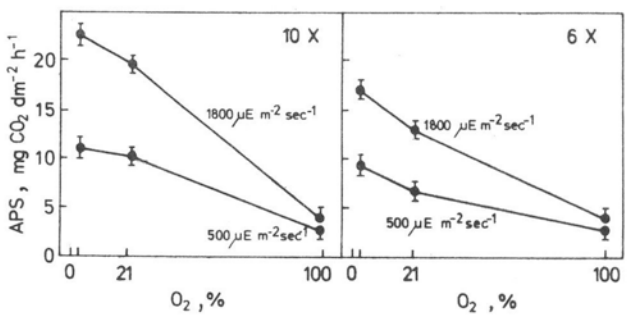

Fig. 1. Rates of apparent photosynthesis of decaploid (10 x) and hexaploid (6 x) tall fescue genotypes as influenced by oxygen concentrations and photosynthetic photon flux density. The bars show the SD

previous reports that decaploid had higher APS compared with hexaploid (Randall et al. 1977, Byrne et al. 1981, Joseph et al. 1981, Wong et al. 1983). Further, the response of APS to O_2 concentration at both PPFDs followed the pattern observed earlier for C-3 types of plants

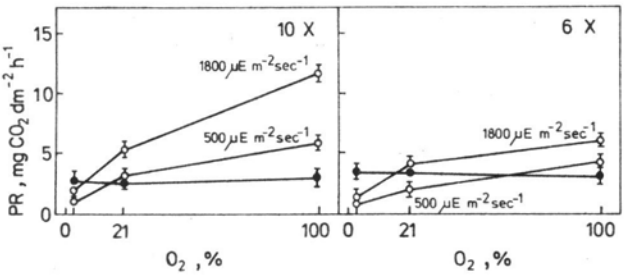

Fig. 2. Rates of photorespiration (open circles) and dark respiration (dark circles) of decaploid (10 x) and hexaploid (6 x) tall fescue genotypes. Reminders as in Fig. 1

(Poskuta 1968). The decaploid also had higher rates of PR than did hexaploid (Fig. 2). At both PPFDs PR was enhanced at 21% O_2 and particulary at 100% O_2 in examined genotypes.

The values of Γ (Fig. 3) were very similar for both genotypes, were independent of PPFD and were a linear function of O_2 concentration. Unlike in mutants of barley (McCashin and Canvin 1979) we did not observe a negative relationship between values of Γ and rates of APS.

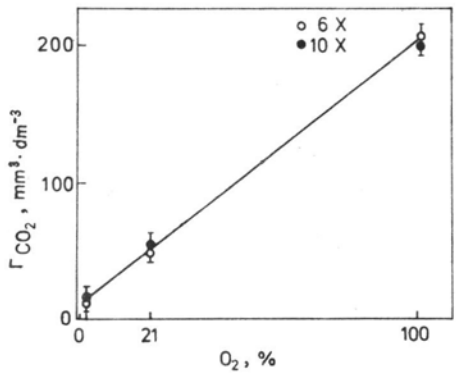

Fig. 3. CO_2 compensation points of decaploid (10 x) and hexaploid (6 x) tall fescue genotypes. Reminders as in Fig. 1

The percentages of PR in true photosynthesis (TPS) were similar for the decaploid and hexaploid genotypes of tall fescue, and followed a straight line relationship with O_2 concentration (Fig. 4). Rates of PR in 21% O_2 were in the range of 18-19% of TPS, and thus were higher than the average 16% reported by Canvin (1979) for C-3 species of plants. However, the percentages were lower than 27% reported by Somerville and Somerville (1983) for a photorespiratory deficient mutant of *Arabidopsis thaliana* where recycling of photorespiratory CO_2 was absent. The reported disparities

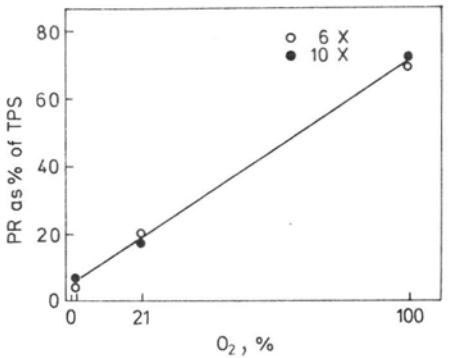

Fig. 4. Percentages of photorespiration in true photosynthesis of decaploid (10 x) and hexaploid (6 x) tall fescue genotypes. Remainders as in Fig. 1

between the percentages of PR in TPS may reflect differences due to species of plant, leaf temperature or an underestimation of PR when determined with an infrared CO_2 analyzer in either a closed or an open system (Canvin 1979) where the magnitudes of CO_2 refixation cannot be measured.

The values of Γ and the percentages of PR in TPS (Figs. 3, 4) were both a linear function of O_2 concentration for both genotypes of tall fescue. Further Γ were not related to rates of APS. These findings indicate that the kinetic properties of RuDP carboxylase/oxygenase *in vivo* were identical in the decaploid and hexaploid genotypes of tall fescue even though their APS is markedly different. These data and conclusions are consistene with reports that ploidy did not alter the kinetic properties of this enzyme in other species (Rejda et al. 1981, McNeil et al. 1981, Meyers et al. 1982, 1983, Molin et al. 1982). In contrast with CO_2 exchange in light, rates of DR were by 16 to 26% lower in the decaploid than in the hexaploid genotype of tall fescue and O_2 had a little effect on DR. Averaged over O_2 percentages applied the ratio of decaploid DR to of hexaploid DR was 0.76 and is similar to the ratio 0.8 for total sucrose content of leaf tissue of these genotypes (Wong et al. 1983). These observations are consitent with the recent data for leaves of wheat and spinach (Azcon-Bieto and Osmond 1983, Azcon-Bieto et al. 1983) which suggest that the concentration of free sugars in the leaves can be a main determinant of rates of DR. The higher rates of DR in the hexaploid may reflect differences between genotypes utilization rates of sucrose. Since CO_2 concentration during light period may affect the rate of DR following short periods of APS (Azcon-Bieto et al. 1983) we measured rates of DR and APS over the same range of CO_2 concentrations 280 to 320 $mm^3 \cdot dm^{-3}$. The possibility exists although probably remote, that when DR was measured after 7 to 10 minutes of darkness the remnants of photorespiratory substrates

were still utilized. Both APS and PR were higher in the decaploid than in the hexaploid (Figs. 1, 2), but their C-3 type plants photosynthetic intermediates were the same (Wong et al. 1983). In contrast, the hexaploid genotype of tall fescue exhibited about a 24% higher rate of DR than the decaploid (Fig. 2) and had about 20% higher content of sucrose (Wong et al. 1983). Tall fescue is known to contain high concentrations of total nonstructural carbohydrates (Jones and Nelson 1979) and rates of DR are not dependent on previous rate of photosynthesis (Volenec et al. 1985). Therefore, DR of tall fescue follows the pattern of this process of pea (Azcon-Bieto et al. 1983). Further, the difference in APS between genotypes of different ploidy level was not due to genetically altered RuBP carboxylase/oxygenase. Apparently other factors such as content of RuBP carboxylase or physical resistances to CO_2 uptake are the major factors causing genotypic differences. These factors were investigated and the results will be presented in the next paper.

Acknowledgement

Financial support came from a Weldon Springs Research Grant to the Plant Biochemistry — Plant Physiology Group at the University of Missouri and from USDA CRGO Grant no 5901 04 109 0366.

REFERENCES

Azcon-Bieto J., Osmond C. B., 1983. Relationship between photosynthesis and respiration. Plant Physiol. 71: 574-581.

Azcon-Bieto J., Lambers H., Day D. A., 1983. Effect of photosynthesis and carbohydrate status on respiratory rates and the involvement of the alternative pathway in leaf respiration. Plant Physiol. 72: 598-603.

Brown R. H., Brown W. V., 1975. Photosynthetic characteristics of *Panicum milioides* a species with reduced photorespiration. Crop Sci. 15: 681-685.

Byrne C. M., Nelson C. J., Randall D. D., 1981. Ploidy effects on anatomy and gas exchange of tall fescue leaves. Plant Physiol. 68: 891-893.

Canvin D. T., 1979. Photorespiration: Comparison between C_3 and C_4 plants. In: Encyclopedia of Plant Physiology, New Series. vol. 6 Gibbs M., Latzko E. (eds.). Springer, Berlin-Heidelberg-New York.

Epstein E., 1972. Mineral nutrition of plants. John Wiley and Sons. New York.

Jones R. J., Nelson C. J., 1979. Respiration and concentration of water-soluble carbohydrates in plant parts of contrasting tall fescue genotypes. Crop Sci. 19: 367-372.

Jordan D. B., Ogren W. L., 1981. A sensitive assay procedure for simultaneous determination of ribulose-1,5 biosphosphate carboxylase and oxygenase activities. Plant Physiol. 67: 237-245.

Joseph M. C., Randall D. D., Nelson C. J., 1981. Photosynthesis in polyploid tall fescue II. Photosynthesis and ribulose-1,5 bisphosphate carboxylase of polyploid tall fescue. Plant Physiol. 68: 894-898.

Keck R. W., Ogren W. L., 1976. Differential oxygen response of photosynthesis in soybean and *Panicum milioides*. Plant Physiol. 58: 552-555.

McCashin B. B., Canvin D. T., 1979. Photosynthetic and photorespiratory characteristics of mutants of *Hordeum vulgare* L. Plant Physiol. 64: 354-360.

McNeil P. H., Foyer C. H., Walker D. A., Bird J. F., Cornelius M. J., Keyes A. J., 1981. Similarity of ribulose 1,5-bisphosphate carboxylases of isogenic diploid and tetraploid ryegrass (*Lolium perenne* L) cultivars. Plant Physiol. 67: 530-534.

Meyers S. P., Nichols S. L., Baer G. R., Molin W. T., Schrader L. E., 1982. Ploidy effects in isogenic populations of alfalfa. I. Ribulose 1,5-bisphosphate carboxylase, soluble protein, chlorophyll and DNA in leaves. Plant Physiol. 70: 1704-1709.

Meyers S. P., Brinegar A. C., Schrader L. E., Jordan D. B., Ogren W. L., 1983. Ploidy effects in isogenic populations of alfalfa (*Medicago sativa* L.). IV. Similarity in physical and kinetic properties of ribulose 1,5-bisphosphate carboxylase/oxygenase. Plant Physiol. 71: 996-998.

Molin W. T., Meyers S. P., Baer G. R., Schrader L. E., 1982. Ploidy effects in isogenic populations of alfalfa. II. Photosynthesis, chloroplasts number, ribulose 1,5-bisphosphate carboxylase, chlorophyll and DNA in protoplasts. Plant Physiol. 70: 1710-1714.

Moser L. E., Volenec J. J., Nelson C. J., 1983. Respiration, carbohydrates content and leaf growth of tall fescue. Crop Sci. 22: 781-786.

Ogren W. L., Hunt L. D., 1978. Comparative study of ribulose 1.5-bisphosphate carboxylase in higher plants. In: Photosynthetic carbon metabolism. Siegelman W., Hind D. (eds.). Plenum Publ. Corp., New York. pp. 127-138.

Ogren W. L., 1984. Photorespiration: pathways, regulation and modification. Ann. Rev. Plant Physiol. 35: 415-442.

Perry S. W., Krieg D. R., Hutmacher R. B., 1983. Photosynthetic rate control in cotton. Plant Physiol. 73: 662-665.

Poskuta J., 1968. Photosynthesis, photorespiration and respiration of detached spruce twigs as influenced by oxygen concentration and light intensity. Physiol. Plant. 21: 1124-1136.

Randall D. D., Nelson C. J., Asay K. H., 1977. Ribulose 1,5-bisphosphate carboxylase. Altered genetic expression in tall fescue. Plant Physiol. 59: 38-41.

Rejda J. M., Johal S., Chollet R., 1981. Enzymic and physicochemical characterization of ribulose 1.5-bisphosphate carboxylase/oxygenase from diploid and tetraploid cultivars of perennial ryegrass (*Lolium perenne* L.). Arch. Biochem. Biophys. 210: 617-624.

Rinehardt C. A., Tingey S. W., Anderson W. R., 1983. Variability of reaction kinetics for ribulose 1,5-bisphosphate carboxylase in barley population. Plant Physiol. 72: 76-79.

Somerville S. C., Somerville C. R., 1983. Effect of oxygen and carbon dioxide on photorespiratory flux determined from glycine accumulation in mutant of *Arabidipsis thaliana*. J. Exp. Bot. 34: 415-424.

Tregunna E. G., Krotkov G., Nelson C. D., 1966. Effect of oxygen on the rates of photorespiration in detached tabacco leaves. Physiol. Plant. 19: 723-733.

Volenec J. J., Nelson C. J., Sleper D. A., 1985. Influence of temperature on leaf dark respiration of diverse tall fescue genotypes. Crop Sci. 24: (in press).

Wong J. H. H., Randall D. D., Nelson C. J., 1983. Photosynthesis in fescue. IV. Carbon assimilation pattern in two genotypes of tall fescue differing in net photosynthesis rates. Plant Physiol. 72: 16-21.

Wpływ stężenia tlenu i natężenia światła na wymianę CO_2 na świetle i w ciemności u dekaploidalnej i heksaploidalnej kostrzewy (Festuca arundinacea Schreb.)

Streszczenie

Badano wpływ stężenia O_2 i natężenia światła na natężenie fotosyntezy netto (APS), fotooddychania (PR), oddychania ciemniowego (DR) oraz na stężenie CO_2 w punktach kompensacyjnych (Γ) dwóch genotypów kostrzewy (*Festuca arundinacea* Schreb.): dekaploidalnej (70 chromosomów) i heksaploidalnej (42 chromosomy). Doświadczenia prowadzono na 2-tygodniowych roślinach rosnących w temp. 25°C i przy natężeniu światła 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Pomiary wykonano stosując zamknięty cyrkulacyjny układ analizatora CO_2 w podczerwieni w świetle o natężeniu 500 i 1800 $\mu mol \cdot m^{-2} \cdot s^{-1}$ i stężeniach O_2 1, 21, 100%. Wykazano, że kostrzewa dekaploidalna w atmosferze 1 i 21% O_2 asymiluje CO_2 z natężeniem od 26 do 46% wyższym w porównaniu z heksaploidalną oraz, że natężenia PR są ściśle skorelowane z natężeniami APS. Wartości Γ były podobne u obu genotypów i były liniową funkcją stężenia O_2 oraz mało zależały od natężenia światła. Procentowy udział PR w fotosyntezie rzeczywistej (TPS = APS+PR) był podobny u obu genotypów i wykazał analogiczne zależności od badanych czynników jak wartości Γ. Natężenie DR wszakże, było od 16 do 26% wyższe u genotypu heksaploidalnego w porównaniu z dekaploidalnym i praktycznie nie zależało od stężeń O_2 i od natężeń APS. Wnioskuje się, że poziom ploidalności u kostrzewy nie ma wpływu na właściwości RuBP karboksylazy/oksygenazy *in vivo* oraz, że PR jest nierozłącznym składnikiem APS.