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Article

Using FACE Systems to Screen Wheat Cultivars for Yield Increases at Elevated CO_2

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Abstract: Because of continuing increases in atmospheric CO_2, identifying cultivars of crops with larger yield increases at elevated CO_2 may provide an avenue to increase crop yield potential in future climates. Free-air CO_2 enrichment (FACE) systems have most often been used with multiple replications of each CO_2 treatment in order to increase confidence in the effect of elevated CO_2. For screening of cultivars for yield increases at elevated CO_2, less precision about the CO_2 effect, but more precision about cultivar ranking within CO_2 treatments is appropriate. As a small-scale test of this approach, three plots, each of four cultivars of wheat, were grown in single FACE and control plots over two years, and the cultivar rankings of yield at elevated and ambient CO_2 were compared. Each replicate plot was the size used in traditional cultivar comparisons. An additional test using four smaller replicate plots per cultivar within one FACE and one ambient plot was used to compare nine cultivars in another year. In all cases, elevated CO_2 altered the ranking of cultivars for yield. This approach may provide a more efficient way to utilize FACE systems for the screening of CO_2 responsiveness.

Keywords: wheat; elevated CO_2; free-air carbon dioxide enrichment; screening; yield

1. Introduction

The CO_2 concentration in the atmosphere continues to increase rapidly, and may increase yields of C_3 crops, helping to meet the expected increased demand for agricultural products. Intraspecific variation in the responsiveness of yield has been reported in many C_3 crops species: barley [1], common bean [2], cow pea [3], oat [4], rape [5], rice [6,7], soybean [8,9], and wheat [10,11]. Such variation could allow the achievement of larger field yield increases as CO_2 rises, if new cultivars could be developed to better exploit the rising CO_2 [12]. However, with the possible exceptions of elevated CO_2 differentially prolonging vegetative growth in soybean [9,13], and increasing leaf mass per area in wheat [14], traits accounting for intraspecific differences in the CO_2 response of yield have mostly not yet been identified. Additionally, many of the studies of intraspecific variation in response were conducted in controlled environment chambers or glasshouses, and tests of whether the ranking of CO_2 responses of yield extrapolates to field conditions have seldom been conducted.

In the field, open top chambers are too small to compare more than just a few cultivars at a time, while free-air carbon dioxide enrichment (FACE) systems can be considerably larger. The two FACE experiments which have compared the largest number of cultivars have been for up to 18 cultivars of soybeans in Illinois [8] and eight cultivars of rice in Japan [6]. The FACE rings were 20 m in diameter for soybean (but only one-half of the plot was used for the 18 cultivars), and 17 m diameter for rice. The sub-plot size per cultivar was 2.3 m^2 for soybeans, and ≥ 3 m^2 in rice, with yield assessed for 1.15 m^2 area in soybeans, and 0.36 to 0.95 m^2 in rice. In these experiments, there were four enriched and four ambient FACE rings, with one subplot of each cultivar in each ring. While this experimental design has replication for the CO_2 effect, variation among rings within each CO_2 treatment would tend to obscure cultivar differences in CO_2 response. More precision about cultivar differences within a CO_2 treatment could be provided by replication of cultivar subplots within a CO_2 treatment plot. This raises the question of the size of the subplots required to accurately rank cultivars for yield within a CO_2 plot. We explored this approach with a comparison of wheat cultivars, first using a subplot size typical of current wheat variety test trials in our region, and in a second experiment using smaller subplots. Yield trials are generally not replicated in the same location and year, but over years and locations, and that approach is also probably best for elevated CO_2 yield trials as well. However, the intent here was not to conduct multi-year yield trials at elevated CO_2, but to test the feasibility of the approach in a FACE system. Once a substantial number of lines with strong and weak yield responses to elevated CO_2 have been identified, identification of traits responsible for such differences in response would be a next step toward selecting crops for future CO_2 environments.

2. Results

In the first experiment, which compared responses of four cultivars in two different years, significant differences among cultivars occurred for seed yield both at ambient and elevated CO_2, at $p = 0.05$ in both years (Table 1). The range of mean yields within CO_2 treatments was 25% to 30% of the overall mean for both years and CO_2 treatments. In the first year, the highest and lowest yielding cultivars, Pioneer 25 R40 and Choptank, respectively, were the same at both CO_2 levels. The second and third ranked cultivars, Pioneer 25 R32 and Jamestown switched rank between CO_2 treatments (Table 1). In the second year, the ranking of the yields Pioneer 25 R32 and Jamestown responded to the CO_2 treatment in the same way as in the first year, that is, the ranking of Jamestown increased at elevated CO_2, and the ranking of Pioneer 25 R32 deceased at elevated CO_2 (Table 1). In the second year, the ranking of Pioneer 25 R32 decreased even below that of Choptank.

Table 1. The mean seed yield (g dry mass m^{-2}) and the ranking of mean yield of four wheat cultivars grown at elevated and ambient CO_2 in 2013 and 2014; the probability of significant differences in yield within a year and CO_2 treatment; and the mean coefficient of variation (CV). Within columns, values followed by different letters were significantly different at $p = 0.05$, by ANOVA.

Year	2013				2014			
CO_2 Treatment	**Ambient**		**Elevated**		**Ambient**		**Elevated**	
Cultivar:	**Rank**	**Yield**	**Rank**	**Yield**	**Rank**	**Yield**	**Rank**	**Yield**
Pioneer 25 R40	1	482 a	1	530 a	1	452 a	1	445 a
Pioneer 25 R32	2	458 ab	3	425 c	2	428 a	4	350 c
Jamestown	3	442 b	2	475 b	3	356 b	2	410 b
Choptank	4	350 c	4	410 c	4	348 b	3	391 b
Probability	0.021		0.028		0.030		0.041	
Mean CV (%)	12.2		9.4		10.1		11.7	

In the second experiment, which compared responses of nine lines in one year, significant differences among lines in yield occurred at both CO_2 levels (Table 2). The range of mean yields among lines was larger relative to the overall mean yield at ambient CO_2 (60%) than at elevated CO_2 (29%). The mean yield of each line at elevated CO_2 was not significantly correlated with its mean yield at ambient CO_2, nor was the ranking of yields significantly correlated for the two CO_2 treatments (Figure 1). The ranking of line 1 increased by three places at elevated CO_2 compared with ambient CO_2, and the ranking of two other lines, 4 and 7, increased by two places at elevated CO_2 (Table 2). The ranking of line 5 decreased by five places at elevated CO_2, line 11 decreased by three places, and line 2 decreased by two places.

Table 2. The mean seed yield (g dry mass m^{-2}) and the ranking of mean yield of nine wheat lines grown at elevated and ambient CO_2 in 2015; the probability of significant differences in yield within a year and CO_2 treatment; and the mean coefficient of variation (CV). Within columns, values followed by different letters were significantly different at $p = 0.05$, by ANOVA. The identity of the nine lines is presented in Table 3.

CO_2 Treatment:	**Ambient**		**Elevated**	
Line:	**Rank**	**Yield**	**Rank**	**Yield**
1	8	353 c	5	513 ab
2	1	567 a	3	538 a
3	2	465 b	1	570 a
4	4	418 b	2	560 a
5	3	430 b	8	460 c
6	7	357 c	6	503 b
7	9	320 c	7	495 bc
8	5	410 b	4	535 a
11	6	365 c	9	423 c
Probability		0.031		0.048
Mean CV (%)		12.4		10.3

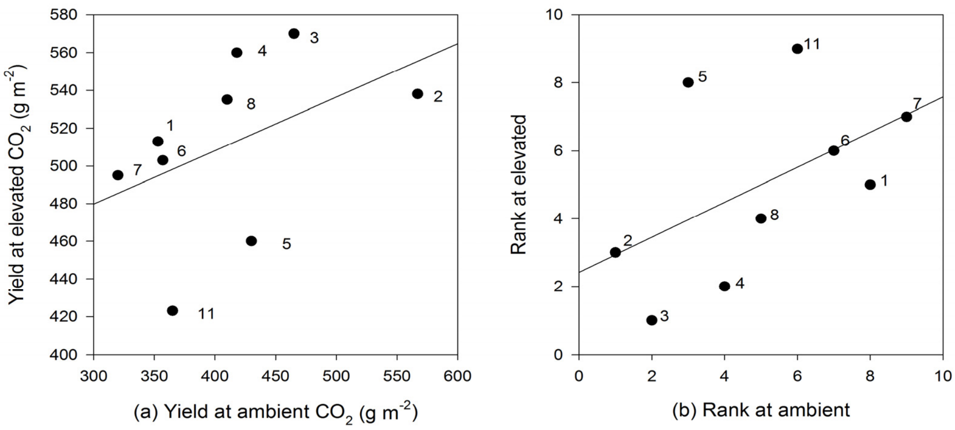

Figure 1. The mean grain yield of nine lines of wheat grown at elevated and ambient CO_2 (**a**), and the ranking of grain yields at elevated and ambient CO_2 (**b**). Linear regressions had an R^2 value of 0.200 for yield, and 0.267 for the ranking of yields, which are not significant at $p = 0.05$. The numbers identify the lines, which are listed in Table 3.

Table 3. The nine lines of wheat tested in the second experiment. All lines are from the wheat association mapping initiative breeding population developed by Centro Internacional de Mejoramiento de Maíz y Trigo [15].

Line Number	**Identification**
1	FCT/3/AZ//MUS/4/DOVE/BUC
2	LEA/TAN/4/TSH/3/KAL/BB//TQFN/5/PAVON/6/SW89.3064
3	MILAN/3/JUP/BJY//URES
4	PRL/SARA/TSI/VEE#5
5	CLC89//ESDA/KAUZ/3/BJY/COC//PRL/BOW
6	KAMBARA2
7	CN079//PF70354/MUS/3/PASTOR/4/BAV92
8	CN079//PF70354/MUS/3/PASTOR/4/CROC_1/AE.SQUARROSA(224)//OPATA
11	CHIH95.1.10

The three lines which had the largest increases in yield ranking at elevated CO_2 in the second experiment (lines 1, 4, and 7) averaged 34% increases in the number of seed heads and 15% increases in seeds per head in the elevated CO_2 plot. The three lines with the largest decreases in yield ranking at elevated CO_2 in this experiment (lines 5, 11, and 2) averaged 7% increases in seed head number, and 1% increases in seeds per head in the elevated CO_2 plot.

The coefficient of variation for averaged 11% across lines within each plot, for both experiments (Tables 1 and 2), that is both for a subplot size of 3.0 m^2 in the first experiment, and 0.20 m^2 in the second experiment. The coefficient of variation averaged the same at ambient and elevated CO_2 in both experiments (Tables 1 and 2). Homogeneity of variance tests also indicated no significant differences in variance among lines in either CO_2 treatment in either experiment.

3. Discussion

The first experiment used a plot size typical of local variety test plots for wheat [16] in Maryland, and indicated that there were significant differences in yield among the four cultivars, both at ambient and at elevated CO_2. However, the comparison of cultivar rankings indicated that Jamestown had a better yield response to elevated CO_2 than did Pioneer 25 R32 in both years, and that Choptank also had a better yield response than Pioneer 25 R32 in the second year. Thus, this experimental design revealed cultivar differences in yield response to elevated CO_2 while using only a single elevated CO_2 plot and a single ambient CO_2 plot. If a few years of local yield trials at ambient CO_2 were available, one might not need a concurrent ambient CO_2 trial in order to determine which cultivars had superior yield responses to elevated CO_2. However, the relatively large size (4 m^2) of the cultivar subplots used in this experiment would greatly limit the number of cultivars that could be compared even using larger FACE systems.

The second experiment tested whether much smaller subplot sizes could also be used to indicate differences among wheat lines in yield responses to elevated CO_2. The lack of increase in variability among subplots for the smaller size, as measured by the coefficient of variation, indicated no loss in precision in comparing yield among lines when using the smaller subplot size. The mean coefficient of variation of 11% for both the larger and smaller sub-plots is similar to typical coefficients of variation in standard wheat yield trials in Maryland [17]. For example, among 57 lines compared at Beltsville in the Maryland State Wheat Trials in the same year as our second experiment, the mean coefficient of variation was 12.8%.

In our second experiment, nine lines were compared in an elevated CO_2 plot of only 36 m^2, with $n = 4$. A FACE ring 20 m diameter could theoretically be used to compare responses of about 90 wheat lines, with the subplot size and number of replicate subplots used here in the second experiment. At this ring size, horizontal uniformity of CO_2 concentration may become an issue [18], and an area distributed FACE system [19], as used here, may be more effective. Identification of lines with larger yield increases at elevated CO_2 is only a first step to improving the response of a crop species to future CO_2 conditions. Identification of traits responsible for differences in yield is an important next step. The preliminary data presented here in the second experiment suggest that both increases in the number of seed heads per m^2 and in the number of seeds per head may be important parameters in yield increases at elevated CO_2 in wheat. The approach used here of having replicated subplots within one elevated CO_2 treatment plot may provide a more efficient route to identifying lines with improved yield responses to elevated CO_2 under open-air field conditions.

4. Materials and Methods

All **e**xperiments were conducted at the South Farm of the Beltsville Agricultural Research Center, Beltsville MD (39°02′ N, 76° 94′ W, elevation 30 m). In one experiment, four locally adapted cultivars of wheat (*Triticum aestivum* L.)—Jamestown, Choptank, Pioneer 25 R40, and Pioneer 25 R32—were grown in one elevated CO_2 plot and one ambient CO_2 plot for two years. In this experiment, each plot was 60 m^2 in area, and there were three subplots of 4 m^2 area of each cultivar, with cultivars randomly assigned to subplots. Row width was 15 cm, and plant density was about 120 plants m^{-2}. The two plots were in the same field, within 50 m of each other, at locations randomly assigned each year. In a second experiment, nine lines from the WAMI breeding population [15] listed in Table 1, selected for differences in tillering, were grown in one elevated and one ambient plot, each 36 m^2 in area. For each CO_2 treatment, there were four subplots, each 0.25 m^2 in area per genotype, arranged randomly. Each subplot had four 12.5 cm wide rows, 50 cm in length, with about 120 plants m^{-2}. The borders of each subplot, and the perimeter 0.75 m of the whole plots were planted with Choptank wheat, which was not sampled. In both experiments, the plots were tilled and planted in mid-October, following soybean crops, and harvested in mid-June. A 10-10-10 nitrogen, potassium, phosphorus fertilizer was applied when regrowth began in spring, at a rate providing 25 g N m^{-2}. No significant pest problems occurred. As is usual for this climate, frequent precipitation prevented any significant soil water deficits.

CO_2 enrichment began at planting, using an area-distributed FACE system, which reduces the horizontal variation in mean CO_2 compared with perimeter ring FACE systems [19]. CO_2 enrichment was applied continuously except when either air or soil temperatures were below 0 °C. The daytime target enrichment was 190 μmol mol^{-1} above the ambient concentration, and 220 μmol mol^{-1} above the ambient concentration at night. These treatments acknowledge that the CO_2 concentrations which crops will experience in the future will probably be more increased at night than during the daytime because of enhanced photosynthesis and respiration per ground area. Mean CO_2 concentrations during periods when temperatures were above 0 °C over the three years of these experiments averaged 422 and 628 μmol mol^{-1} for the ambient and elevated plots, respectively. The midday ambient CO_2 concentration averaged 392 μmol mol^{-1}.

The crops were harvested at grain maturity. In the experiments with the 4 m^2 subplots, total above ground dry mass, head number, tiller number, mean mass per grain, and total grain dry mass were obtained from a bordered 3 m^2 area within each subplot. In the experiment with 0.25 m^2 subplots, bordered 0.20 m^2 areas were harvested from each subplot, with the same plant parameters measured.

Because there was only one replicate plot of each CO_2 treatment each year, no assessment of the overall CO_2 effect was made in either experiment. Using the replication of the genotype subplots within each CO_2 treatment, analysis of variance was used to test whether genotypic differences in yield existed within each plot, separately for each CO_2 treatment and year. The coefficient of variation (the standard deviation divided by the mean × 100%) averaged across genotypes was compared for the experiments with the large and the small subplot sizes. The coefficient of variation should increase when plots become too small relative to the spatial variation in environmental properties. Homogeneity of variance tests were also conducted among lines within each CO_2 treatment in both experiments. An altered ranking of genotype yields at ambient versus elevated CO_2 within a year was taken as preliminary evidence of a differential response to CO_2 enrichment among genotypes. In the experiment with two years of observations, we examined the consistency of changes in rank with CO^2 across years. A high correlation among lines for yield or the ranking of yield at ambient vs. elevated CO_2 could indicate little variation among lines in their CO_2 response, so these correlations were examined in the second experiment, which had only one year of yield data.

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Abbreviations

The following abbreviations are used in this manuscript:

FACE	Free-air carbon dioxide enrichment

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