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RESEARCH LETTER

Yield component responses of biotechnology-derived drought tolerant maize under controlled environment conditions

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Abstract

Drought stress is a prevalent environmental factor that results in significant yield losses for maize (*Zea mays* L.). Genetic improvements through modern breeding methods and the introduction of biotechnology-derived traits have been used to improve tolerance to drought stress. Conducting yield efficacy trials in the field for plants that exhibit tolerance to abiotic stress poses several challenges, including the inability to control multiple unpredictable environmental stressors. Controlled environment conditions were tested to evaluate yield parameters commonly observed in the field for MON 87460 maize expressing the cold shock protein B (CspB) protein. Maize plants subjected to water-limitation treatment in the controlled environment exhibited phenotypic characteristics of drought stress consistent with those observed under field conditions. MON 87460 exhibited significant relative increases in ear length, kernel number, and grain weight following water-limitation treatment. These results demonstrate that the controlled environment is an alternate option for evaluating drought tolerance in maize.

1 | INTRODUCTION

Among the environmental stresses exerted on crops, drought stress has been the most prevalent, resulting in significant economic losses (Boyer, 1982). In recent years, biotechnology-derived traits have been used to improve crop tolerance to drought stress (Bruce, Edmeades, & Barker, 2002; Castiglioni et al., 2008). Maize (*Zea mays* L.) hybrid MON 87460 (Bayer) expresses cold shock protein B (CspB) from *Bacillus subtilus* that confers drought tolerance in maize resulting in reduced yield loss under water-limited conditions (Castiglioni et al., 2008; Nemali et al., 2015; Sammons, Whitsel, Stork, Reeves, & Horak, 2014). Field studies have shown that improvement in yield performance by MON 87460 under water-limited conditions is attributed to increased harvest index or ratio of grain yield to total plant dry weight (Nemali et al., 2015). Plants containing event MON 87460 produced more kernels per ear compared with controls, which was associated with increased ear size at silking. Similar to other published research, these results also indicate that improved grain yield in maize under water limitation can, in part, be driven by

Abbreviations: ASI, anthesis–silking interval; SA, standard agronomic; WL, water limited.

relatively larger ears and increased dry-matter partitioning to ears, leading to increased kernel set (Fisher & Palmer, 1983; Severini, Borrás, Westgate, & Cirilo, 2011). This is particularly relevant when considering water-limitation scenarios that occur preflowering when characteristics such as ear size and kernel set that drive yield component responses are determined (Abendroth, Elmore, Boyer, & Marlay, 2011; Fisher & Palmer, 1983).

Field trials are commonly used to evaluate the performance of traits conferring drought tolerance. Appropriate locations to conduct such trials are limited and are also subject to governmental regulation that can significantly limit or even prohibit field testing of transgenic crops (Fedoroff et al., 2010). Moreover, variation in environmental factors such as high heat, extreme drought, and rainfall during critical periods of crop development can potentially impact the ability to assess the effect of introduced traits conferring drought tolerance. Alternatively, conducting such trials in the controlled environment offers the advantage of running multiple trials per growing season, allowing for selective application of drought stress at critical growth stages while potentially minimizing confounding effects from other biotic and abiotic stressors present in field trials (Grant, Jackson, Kiniry, & Arkin, 1989; NeSmith & Ritchie, 1992).

Despite several advantages, controlled environment testing for trait efficacy is limited in crop improvement programs. This is in part due to the misconception that the observed responses under controlled environment trials are specific to the testing environment and may not be representative of field conditions. Moreover, a limited number of studies in maize have been conducted in controlled environments where plants were grown up to harvest stage as most of the research has been limited to vegetative stages. This is mainly the result of difficulties associated with cross-pollination (due to lack of sufficient air movement) and subsequent seed set in maize grown in controlled environments.

The objective of this study was to evaluate the utility of the controlled environment for drought efficacy testing in maize by subjecting container-grown MON 87460 and a near-isogenic conventional control to water-limited conditions in a greenhouse and comparing physiological and yield components differences between them.

Core Ideas

- MON 87460 maize exhibits reduced yield loss under drought field conditions.
- Inability to control for multiple stressors poses challenges to field efficacy trials.
- Greenhouse drought responses were comparable to published field studies with maize.

2 | MATERIALS AND METHODS

2.1 | Plant materials and cultivation

The greenhouse trial was conducted from July through November 2016. Plant materials tested included drought-tolerant 110-d maturity transgenic maize MON 87460 and a genetically matched near-isogenic conventional control. Plants were started from seed in 30.5-cm (15-L) plastic pots (Hummert International) filled with a soilless substrate (Fafard 3B, SunGro Horticulture) and supplemented with Osmocote fertilizer (19–6–12 N–P–K, Scotts Co.) at a rate of ∼3.6 kg m^{-3}. An electronically controlled drip irrigation system was used to supply water to individual pots via capillary tubing and drip emitters. Before the trial, the system was calibrated to deliver desired volumes to individual pots. Plant growth stages were monitored using the Leaf Collar method (Abendroth et al., 2011). At approximately the V6 growth stage, plants were supplied with 15–2.2–12.5 N–P–K water-soluble fertilizer (Peters Excel 15–5–15 Cal-Mag Special, Everris NA Inc.) that contained N at a rate of 150 mg L^{-1}. In addition, approximately 45 g of gypsum (United States Gypsum Company) was incorporated into the substrate thorough the drip-system irrigation. Plants were watered uniformly and as necessary with approximately 2000 ml water pot^{-1} until the V8 vegetative stage, at which the water-limitation treatment was imposed (see below). To address seasonal impacts on maize growth, supplemental lighting using high-pressure sodium lights was provided during growth to provide additional light when ambient light levels in the greenhouse fell below 500 μmol m^{-2} s^{-1}. Supplemental lighting was provided daily as needed between 4:00 a.m. and 8:00 p.m. for a 16-h photoperiod.

2.2 | Experimental design

There were two concurrent but separate experiments including a water limited (WL) and a standard agronomic (SA) treatment. The SA experiment was used to measure yield drag, if any, in MON 87460 and qualitatively compare the extent of drought stress experienced by isogenic control plants in the WL treatment. Plants in both experiments were arranged in a randomized complete block design. There were 42 replications in the WL experiment and 12 replications within SA experiment. The number of replications needed in the WL experiment was estimated from a power analysis using data from a previous study (data not shown). Each replication consisted of two pots, one planted with MON 87460 and one planted with the conventional control. Within each replication, both MON87460 and the control were randomly assigned to one of the two positions. Additional conventional maize plants were cultivated around the perimeter of the greenhouse with staggered planting dates to ensure enough pollen was available during the entire interval of silking.

2.3 | Drought stress imposition

Before the study, the irrigation system was calibrated to deliver a desired volume of water to each pot per unit of time. The irrigation system was run and water from each emitter was collected in empty bottles. The mean observed pot-to-pot variation in volume of water delivered was 9.8%. Before imposition of drought stress, all plants in the greenhouse were provided 2000 ml water pot^{-1} whenever the soil media surface appeared dry. When approximately 50% of all plants reached the V8 growth stage, the water-limitation treatment was imposed on plants in the WL experiment until 50% of plants reached the R1 stage. During water limitation, all pots in the WL experiment received 1200 ml pot^{-1} whenever plants in the experiment showed severe leaf curl symptoms. The growth-stage interval selected to apply water-limitation treatment in our study corresponds with the time in maize development when the kernel set is determined (Abendroth et al., 2011). Plants in the SA experiment continued to receive 2000 ml water pot^{-1} throughout the treatment phase when the top of the surface of the substrate appeared dry. From the conclusion of the water-limitation treatment phase until R5, plants in both SA and WL experiments were maintained by providing 2000 ml water pot^{-1} as needed (approximately every 2–3 d). Pots were allowed to dry down after the R5 growth stage. Because plants in the WL and SA experiments were watered at different volumes and frequencies during the water-limitation treatment, the applied watering regimen resulted in a relative overall water-deficit in the WL experiment of 19% compared with the SA experiment.

2.4 | Pollination

To ensure optimal pollination of all receptive silks occurred following drought-induced delays in silking, each ear was pollinated by hand. Each day during the flowering stage, pollen was collected from all plants exhibiting visible pollen shed. Collected pollen was then applied to exposed silks with the aid of a paintbrush. This was repeated daily such that each ear was pollinated at least twice within 5 d of the first appearance of silk extrusion; the daily pollinations continued until pollen shed in the greenhouse was complete.

2.5 | Measurements

Recorded environmental conditions were appropriate for cultivation of maize in a controlled environment (see Supplemental Figure S1). Anthesis–silking interval (ASI) was determined with daily counts of the total number of plants shedding pollen and silking. The ASI for each experiment was defined as the difference in days from when 50% of the plants within the experiment were shedding pollen to when 50% of plants showed evidence of silk extrusion. Ear length was measured as the distance from the base to the tip of the protruded ear (directly without removal of ears from the plant) at the beginning of the R2 growth stage, since ears typically reach their maximum length by the end of R1 (Abendroth et al., 2011). Yield component values, including total grain weight per plant and kernel number per plant, were measured at harvest. At maturity, harvested ears from all plants were removed from the stalk by hand, husks were removed, and ears were dried in a forced air oven at 80 ^{o}C until <1% change in weight was observed between two daily measurements of representative ears. Dried ears were shelled and separated kernels were counted with a calibrated seed counter (Agriculex) to determine the number of kernels per ear (kernel number) and weighed with an analytical balance (Mettler-Toledo) to determine grain weight per ear. Single kernel weight was calculated by dividing the grain weight per ear by the kernel number per ear.

2.6 | Statistical analysis

Pairwise comparisons between materials were defined within the ANOVA and tested using t-test (SAS, Version 10.1, SAS Institute, Cary, NC). A predetermined alpha level of .05 was set for any comparison to be statistically significant.

3 | RESULTS AND DISCUSSION

Plants subjected to the water-limitation treatment received approximately 19% less water relative to those in the SA experiment. This indicates that plants in the WL treatment were subjected to mild-to-moderate level of drought stress in our greenhouse trial. Maize subjected to drought stress in the field exhibits physiological responses including leaf curl and increased ASI (Bänziger, Edmeades, Beck, & Bellon, 2000; Bruce et al., 2002; Uribelarrea, Cárcova, Otegui, & Westgate, 2002). Administration of preflowering drought stress on plants in the WL experiment resulted in observable leaf curl as well as a 2.8 d increase in mean days-to-silk compared with those in the SA experiment (55.6 ± 0.28 d WL vs. 52.8 ± 0.71 d SA; mean ± SE). In contrast, water limitation had no

TABLE 1 Each ear was evaluated for grain yield components. Results are presented as mean values and standard error of the mean for each parameter is provided in parentheses. The percentage change is defined as the percentage change in MON 87460 compared with the conventional control for a given parameter

Experiment	Characteristic	Conventional	MON 87460	% Change	*P*-value[a]
Standard agronomic ($n = 12$)	Ear length	26.0 (0.9) cm	26.0 (0.8) cm	0.0	n.d.
	Total kernel number	522.5 (28.4)	536.3 (26.7)	2.6	n.d.
	Single kernel wt.	380.2 (12.9) mg	388.5 (20.2) mg	2.2	n.d.
	Grain wt. per ear	200.6 (10.6) g	214.6 (10.7) g	7.0	n.d.
Water-limited ($n = 42$)	Ear length	24.1 (0.3) cm	24.8 (0.3) cm	2.9	0.040
	Total kernel number	456.4 (15.7)	497.4 (11.7)	8.9	0.018
	Single kernel wt.	399.9 (5.6) mg	392.6 (4.1) mg	−1.8	0.182
	Grain wt. per ear	183.3 (4.8) g	197.6 (3.4) g	7.8	0.001

[a]*P*-value ≤ .05 was considered statistically significant; n.d., not done.

effect on the time to anthesis, which is consistent with physiological responses observed in maize subjected to drought stress under field conditions (Araus, Serret, & Edmeades, 2012). The increased time to silk under water-limiting conditions resulted in an increased ASI of 5 d for plants in the WL experiment compared with 2 d in the SA experiment.

Following drought stress, ears from conventional plants in the WL treatment exhibited numerical reductions in ear length (7.3%), kernel number (12.8%), and grain weight (8.6%) compared with those under SA conditions (Table 1). The magnitude of this reduction in yield component parameters is similar to those observed in other water-limitation maize trials conducted in controlled environments and field trials (Grant et al., 1989; Nemali et al., 2015; NeSmith & Ritchie, 1992). In the SA experiment, that is, absence of water limitation, MON 87460 and the conventional maize exhibited comparable yield component endpoints including ear length, total kernel number, single kernel weight, and grain weight (Table 1). Under water-limited conditions, ears from MON 87460 plants exhibited a 2.9% increase in ear length, 8.9% increase in the number of kernels per ear, and a 7.8% increase in total grain weight compared with the conventional control. In contrast, there were no statistical differences in single kernel weight between MON 87460 and conventional control. The magnitude of the yield component parameters under water-limited conditions in the greenhouse are similar to what has been observed in the field drought trials for both MON 87460 and conventional maize (Nemali et al., 2015). These results strongly support that MON87460 maize subjected to water-limitation stress under controlled environment conditions (greenhouse) displays physiological and productivity responses consistent with those under drought stress.

Inspection of the results indicates that the observed relative increase in grain weight from MON 87460 was the result of an increase in kernel number per ear as opposed to single kernel weight. This is consistent with observations from field trials with MON 87460 that showed kernel number as opposed to single kernel weight was more closely correlated with grain weight (Nemali et al., 2015). In the field, yield losses due to water deficits that occur preflowering when kernel sink capacity is set are typically associated with a decrease in kernel number as opposed to individual kernel weight (Abendroth et al., 2011; Bolaños & Edmeades, 1996; Boyer & Westgate, 2004; Campos et al., 2006; Nemali et al., 2015; Ouattar, Jones, & Crookston, 1987). Grant and colleagues imposed multiple overlapping intervals of water-limitation treatments on maize under greenhouse conditions. Results from these trials indicated that stress applied during flowering resulted in reductions in kernel number and grain weight while stress applied during late grain fill preferentially reduced grain weight (Grant et al., 1989). Similarly, when water was withheld from field-cultivated maize during preflowering growth stages with the use of a rain shield, a reduction in kernel number per ear was observed (NeSmith & Ritchie, 1992). While differences in regional climate, available sunlight, and plant genetics may affect the degree of water limitation needed to elicit physiological responses in maize, the results presented in this trial are consistent with those observed in the field as well as other controlled environment trials.

In conclusion, the established greenhouse assay reproduced drought-associated physiological responses in maize including increased ASI and reduced productivity. Consistent with application of drought stress during preflowering growth stages, the observed increase in grain weight by MON 87460 compared with the conventional control correlated with increased ear length and kernel number per ear with response magnitudes comparable to those observed in field trials. These results validate the use of controlled environment conditions to evaluate maize products for drought tolerance and provide a potential testing alternative in regions where open field trials are not feasible or permitted.

CONFLICT OF INTEREST

All authors, with the exception of K.N., are employees of Bayer CropScience.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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