Plant nuclear factor Y (NF-Y) B subunits confer drought tolerance and lead to improved corn yields on water-limited acres

Donald E. Nelson*[†], Peter P. Repetti[‡], Tom R. Adams*, Robert A. Creelman[‡], Jingrui Wu*, David C. Warner*, Don C. Anstrom*, Robert J. Bensen*, Paolo P. Castiglioni*, Meghan G. Donnarummo*, Brendan S. Hinchey*, Roderick W. Kumimoto[‡], Don R. Maszle[‡], Roger D. Canales*[‡§], Katherine A. Krolikowski[‡], Stanton B. Dotson*, Neal Gutterson[‡], Oliver J. Ratcliffe[‡], and Jacqueline E. Heard*[‡§]

*Monsanto Company, 62 Maritime Drive, Mystic, CT 06355; and [‡]Mendel Biotechnology, Inc., 21375 Cabot Boulevard, Hayward, CA 94545

Commercially improved crop performance under drought conditions has been challenging because of the complexity of the trait and the multitude of factors that influence yield. Here we report the results of a functional genomics approach that identified a transcription factor from the nuclear factor Y (NF-Y) family, *AtNF-YB1*, which acts through a previously undescribed mechanism to confer improved performance in *Arabidopsis* under drought conditions. An orthologous maize transcription factor, ZmNF-YB2, is shown to have an equivalent activity. Under water-limited conditions, transgenic maize plants with increased ZmNF-YB2 expression show tolerance to drought based on the responses of a number of stress-related parameters, including chlorophyll content, stomatal conductance, leaf temperature, reduced wilting, and maintenance of photosynthesis. These stress adaptations contribute to a grain yield advantage to maize under water-limited environments. The application of this technology has the potential to significantly impact maize production systems that experience drought.

Arabidopsis | maize | transgenic | transcription factor

Improved productivity under periodic drought stress is a major challenge for global agriculture. Increasing the yield of agricultural crops grown under drought conditions is challenging because of the low heritability of the trait, the unpredictable nature of most periods of drought stress encountered in growing areas, and gaps in our understanding of drought biology (1, 2). As a consequence, new approaches were sought for improving the performance of crops grown under periodic drought conditions (3–6). We used a functional genomics strategy, with the *Arabidopsis* model system, to identify molecular regulators of drought stress adaptation in plants. The *Arabidopsis* findings are shown to be directly applicable to commercial crop improvement.

Maize crops are impacted by drought throughout the life cycle, with the greatest losses being observed when stress occurs in the development phase just before and after flowering (7). During periods of decreased water availability, plants often exhibit stress symptoms, including increased leaf senescence and cellular damage due to photooxidative stress, as well as reduced leaf expansion, carbon fixation, and negative effects on reproductive development. Together, these processes result in an overall decrease in the total carbon assimilated per plant and reductions in grain yield (8–10).

Integration of multiple stress response pathways during these critical vegetative and reproductive development windows is an effective strategy to significantly impact crop performance under field conditions. Transcription factors can be used to elicit multiple biochemical and developmental pathways that regulate drought tolerance, thereby improving performance during drought. The best stress-responsive transcription factors are the C-repeat-binding factor (CBF)/dehydration-responsive element-binding (DREB)

proteins that belong to the AP2/ethylene-responsive element-binding protein family (11, 12). These factors enhance or modulate the expression of genes with a CBF/DRE box in their promoters and define a major stress tolerance pathway, in addition to the abscisic acid (ABA) biosynthesis/response pathway. However, although increased expression of CBF/DREB proteins has been shown to confer drought tolerance in a growing number of plant species (13), other less-desirable developmental phenotypes, such as stunted growth, are often associated with high constitutive expression. The use of drought-responsive promoters to induce CBF/DREB expression under drought stress conditions has increased the specificity for drought tolerance vs. other undesirable developmental phenotypes (14). Further examples of stress-responsive factors that condition dehydration tolerance have been reviewed recently (15).

Because there are many mechanisms by which plants can tolerate drought, and because transcription factors are well established as regulators of genetic pathways, we reasoned that additional transcription factors would exist that modulate drought responses. A genome-wide systematic analysis of *Arabidopsis* transcription factor families (16) was conducted to identify genes that improve tolerance to environmental stress. A selection of transcription factors was discovered from a range of families that condition enhanced abiotic stress tolerance when constitutively expressed in *Arabidopsis*. Here, the focus is on the role in eliciting drought tolerance of one of these transcription factors, *AtNF-YB1*, a subunit of the nuclear factor Y (NF-Y complex, also known as the HAP or the CAAT family), which mediates transcriptional control through CCAAT DNA elements.

NF-Y is a conserved heterotrimeric complex consisting of NF-YA (HAP2), NF-YB (HAP3), and NF-YC (HAP5) subunits (17). Fungi and animals use single genes to encode each protein subunit. NF-Y transcription factors typically act in concert with other regulatory factors to modulate gene expression in a highly controlled manner. In plants, NF-Y genes have been amplified with ≈10 different genes encoding each subunit

of the transcription factor complex (18–20). Amplification of the family raises the possibility that new and divergent functions of heterotrimeric complexes have evolved in plants.

We identified an NF-YB protein from *Arabidopsis thaliana* and an orthologous NF-YB protein from *Zea mays* that coordinate plant responses to drought tolerance. Our data suggest functional conservation of the underlying drought tolerance pathway across the dicot and monocot lineages. Drought tolerance has been obtained in field trials with maize lines constitutively expressing the ZmNF-YB2 protein, demonstrating the potential of this strategy for improving drought tolerance in commercial crop plants.

Results

Drought Tolerance of *Arabidopsis AtNF-YB1* Constitutive Expression Lines. *AtNF-YB1* (AT2G38880) was identified as a putative regulator of drought-related stress tolerance in a large-scale functional genomics program performed in *Arabidopsis*. Briefly, the effects of constitutive expression of >1,500 putative transcription factor genes from *Arabidopsis* were examined during this screen (R.A.C., R.W.K., O.J.R., and J.E.H., unpublished results). For each transcription factor, multiple independently transformed lines were established and subjected to a panel of plate-based surrogate drought assays including mannitol, PEG, salt, sugar, ABA, heat, cold, and severe dehydration treatments. Transcription factor constitutive expression lines that exhibited a good performance in one or more of these assays were then subjected to more extensive carefully controlled drought assays under soil grown conditions. *AtNF-YB1* was one of ≈40 different transcription factor genes that were confirmed as regulators for enhancement of drought tolerance during this work.

Transgenic *Arabidopsis* plants that constitutively express *AtNF-YB1* were obtained by transforming Col-0 wild-type plants with the cDNA version of the gene under the control of an enhanced 35S promoter. 35S::*AtNF-YB1* lines were visually wild type in their morphology except for a slight but reproducible delay in flowering, which typically occurred 1–6 days late. For example, under continuous light conditions, plants from a representative line, no. 3, showed visible flower buds after an average of 31.1 days vs. 25.7 days for the control plants. Initial screening identified several independent 35S::*AtNF-YB1* lines that showed slightly more vigorous germination than controls under conditions of osmotic stress (9.4% sucrose and 14% polyethylene glycol; data not shown). Based on the results from osmotic stress screens, 35S::*AtNF-YB1 Arabidopsis* lines grown in soil were subjected to drought survival assays. 35S::*AtNF-YB1* plants typically exhibited less severe wilting than wild-type plants after a severe drought period (Fig. 1). Six of eight independent lines tested showed an increased rate of survival compared with controls ($P < 0.1$) upon re-watering following drought (Table 1).

To gain an understanding of the physiological basis for the enhanced survival rate, a representative line (no. 3) was chosen for further in-depth analysis. Transgenic and control plants were grown side by side in flats to the early reproductive stage of development, after which time a drought stress treatment was applied to one-half of the plants. Physiological measurements were made on droughted plants displaying leaf wilting. During drought treatment, transgenic plants maintained higher water potential and photosynthesis rates (measured under saturating CO_2 conditions) than controls, both of which are key phenotypes related to plant productivity (Fig. 2).

The above results demonstrated that an increase in *AtNF-YB1* activity was sufficient to increase tolerance to water deprivation in *Arabidopsis*. We therefore conducted expression profiling experiments on the 35S::*AtNF-YB1* lines to determine whether the protein was acting through a previously recognized molecular response pathway.

Fig. 1. Constitutive expression of *AtNF-YB1* confers drought tolerance in *Arabidopsis*. Representative pots of transgenic plants (*Left*) and controls (*Right*) are shown at the end of a dry-down period (*Upper*) and at 5 days after rewatering (*Lower*).

Microarray Analysis of 35S::*AtNF-YB1* *Arabidopsis* Lines. A "full-genome" baseline transcript profiling experiment was performed on nonstressed whole-rosette tissue from 35S::*AtNF-YB1* seedlings grown under 24-hr light. Surprisingly, relatively few genes were represented in the profile; even when a low stringency cutoff was applied, only 108 of ≈23,000 distinct genes were differentially expressed relative to wild-type controls [nonfalse discovery rate-corrected data, $P < 0.01$; supporting information (SI) *Methods* and SI Table 3]. The small size of the profile likely indicated that *AtNF-YB1* acts near the end of a response pathway, or that its effects were due to constitutive or misexpression in specific tissues or at particular developmental or temporal stages. Gene ontology analysis at the "biological process" level indicated that the most overrepresented processes in the 35S::*AtNF-YB1* profile were related to polysaccharide metabolism, in various forms. However, none of the categories were obviously related to stress tolerance.

To assess whether the mechanism of drought tolerance in 35S::*AtNF-YB1* plants is novel, we made comparisons between the 35S::*AtNF-YB1* profile and the profiles from ABA-treated wild-type plants and from CBF4 transcription factor-expressing plants, each of which have well known drought-tolerance pathway genes induced (21). The genes regulated by constitutive expression of *AtNF-YB1* did not significantly overlap with those regulated in response to ABA treatment nor those expressed in 35S::*CBF4* plants (which show strong correlation with known drought/stress-response pathways). The heat map in Fig. 3 shows the expression pattern for all genes significantly ($P < 0.01$ uncorrected) expressed in either 35S::*AtNF-YB1* or 35S::*CBF* untreated seedlings (compared with wild type), as well as how those genes responded at 4 h after ABA treatment in wild-type seedlings (compared with a mock treatment). Although a significant set of genes was similarly induced by ABA and CBF4 constitutive expression (see SI Table 3), no such correlation was found with the expression profile of 35S::*AtNF-YB1*.

To confirm that *AtNF-YB1* was not acting through previously characterized drought-response mechanisms even during water

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Table 1. Survival of 35S::AtNF-YB1 *Arabidopsis* lines in a soil drought assay relative to wild type

Line	Mean survival ratio*	P value ANOVA[†]	Number of experiments
3	1.3	0.004	23
6	2.1	0.032	3
1,703	1.7	0.080	1
1,704	1.0	1.000	1
1,707	1.4	0.017	3
1,715	1.4	0.150	2
1,720	1.7	0.070	1
1,948	1.7	0.020	2

*Geometric mean over planting dates of proportion of plants surviving in line vs. proportion of plants surviving in wild type.
[†]P value of significance of difference in the transgenic effect calculated in a two-way interaction analysis of variance model (transgene and planting date) with random effects over repeats of the experiment (planting dates).

deficit, several component genes from the CBF and ABA response pathways were selected (see SI Table 4), and we examined their expression by RT-PCR in an independent experiment on 35S::*AtNF-YB1* and wild-type *Arabidopsis* grown under either well watered or droughted conditions (see SI Methods). None of the ABA pathway markers (*CBF4*, *RD29B*, or *RAB18*) or CBF pathway markers (*COR15B*, *KIN1*, and *LEA76*) showed significant and consistent differences in expression between 35S::*AtNF-YB1* plants and controls (data shown as SI Fig. 7). Thus, based on these markers, *AtNF-YB1* does not transcriptionally regulate either the CBF or ABA drought tolerance pathways.

***AtNF-YB1* Has a Native Role in Drought Responses.** Under normal well watered conditions, *AtNF-YB1* is expressed across a range of different organs and tissue types in wild-type plants (19, 20). To determine whether *AtNF-YB1* is part of a native drought-response pathway, quantitative RT-PCR was used to examine the expression profile of the gene in mature leaves from soil-grown wild-type plants harvested over a drought time course. *AtNF-YB1* transcript levels showed a highly significant increase of ≈12-fold (≈3.5 PCR cycles) under severe drought relative to well watered conditions and then returned to basal levels by 24 h after rewatering (Fig. 4). Thus, *AtNF-YB1* is a component of a previously unrecognized transcription-regulated response to water deprivation.

Fig. 2. *Arabidopsis* transgenic lines have improved drought physiology. Water potential (*Upper*) and photosynthesis measured under saturating CO_2 conditions (*Lower*) of *Arabidopsis* plants measured during a stress treatment applied at the early reproductive stage of development.

Collectively, these initial results for *AtNF-YB1* in *Arabidopsis* indicated an evolved function for the NF-YB transcription factor family and suggested a new molecular strategy for enhancing crop drought tolerance. We therefore examined whether a comparable mechanism was present, and useful for enhancing drought tolerance, in maize.

Drought Tolerance of *OsRACT::ZmNF-YB2* Maize Lines. Bioinformatic analysis was used to identify maize homologs of *AtNF-YB1*. One of these homologs, ZmNF-YB2, was selected for constitutive expression studies in maize. A phylogenetic analysis comparing this maize protein with 10 different *Arabidopsis* NF-YB proteins (19, 20) revealed that ZmNF-YB2 is most closely related to *AtNF-YB1* (SI Fig. 8). Furthermore, like *AtNF-YB1*, *ZmNF-YB2* is expressed broadly across maize tissues (data not shown).

ZmNF-YB2 was used to transform an elite maize inbred. The recombinant DNA constructs comprised a rice actin 1 constitutive promoter and a rice actin 1 intron linked to the maize gene. Transgenic maize lines were tested in a greenhouse drought assay, with plants being subjected to 10 days of drought, followed by 3 days of full-water recovery, followed by 9 days of drought, and finally full watering. After 2 days of full watering, the plants that had been subjected to two drought stress treatments usually began to show signs of recovery from a wilted state. However, in some cases, recovery took up to 5–6 days of full watering, and some individual plants never recovered. On average, the recovered transgenic plants were significantly greener and more rapidly reestablished growth than the recovered wild-type plants. The transgenic lines also exhibited less wilting, indicating increased turgor pressure and delayed onset of senescence compared with controls (Fig. 5A). Four independent lines showed improved drought tolerance in greenhouse experiments. These results indicated evolutionary conservation of a novel function within the NF-YB family and suggested the existence of common stress-response pathways in maize and *Arabidopsis*.

Field studies were conducted to investigate the relationship between stress tolerance and enhanced productivity by subjecting *OsRACT::ZmNF-YB2* transgenic maize lines and negative segregant controls to drought conditions. Water was withheld from half of the planted field area during the late vegetative stage; under drought conditions, the transgenic maize plants constitutively expressing ZmNF-YB2 were healthier than the wild-type plants with less leaf rolling (Fig. 5B) and exhibited the following phenotypes: a higher chlorophyll index, higher photosynthesis rate, cooler leaf temperature, and higher stomatal conductance (Table 2). The physiological data represent the combined analysis of six independent lines. Under conditions of ample water supply, *OsRACT::ZmNF-YB2* transgenic maize lines were darker green, flowered 1–3 days earlier than the controls, and had slightly compressed internodes. All other standard agronomic traits, such as early stand, disease ratings,
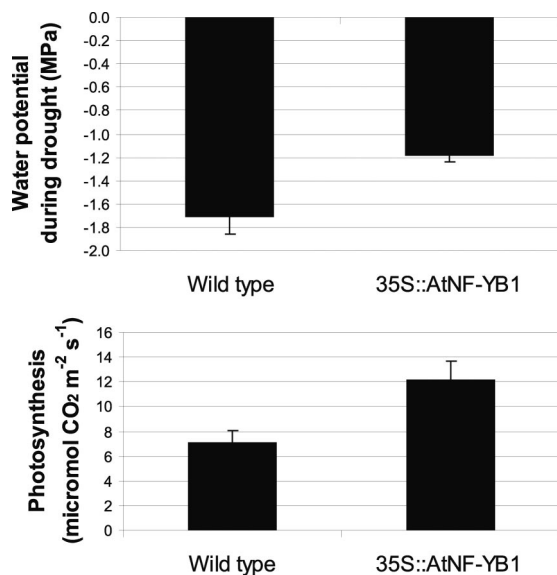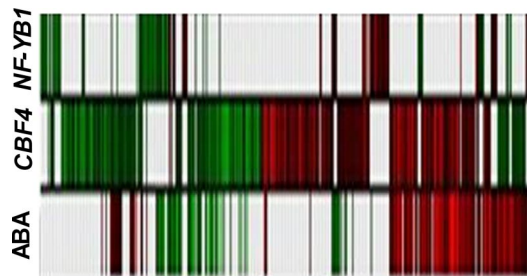
Fig. 3. Agglomerative cluster of all genes (minus hybridization controls) that are differentially expressed in the seedling profiles from 35S::*CBF4* plants or 35S::*AtNF-YB1* (vs. wild-type) plants, as well as the regulation of those genes at 4 h after treatment with ABA in wild type (vs. mock-treated wild-type plants). Red indicates induction, and green indicates repression. White bars indicate that the gene did not display a significant change in expression ($P < 0.01$). An ordered list of the genes included in the heatmap and the corresponding expression data can be found in SI Table 3.

occurrence of lodging, and grain quality, were not different as compared with the negative segregant controls (data not shown).

In field efficacy trials, grain yield was measured under controlled drought treatments; the imposed drought stress resulted in a >50% yield reduction in the negative controls compared with what is expected under fully watered normal conditions. In Year 1, stress levels were notably higher than in Year 2, with negative controls achieving a base yield of 74 bushels per acre (4.6 metric tons per hectare) in Year 1 and a base yield of 102 bushels per acre (6.4 metric tons per hectare) in Year 2 (Fig. 6). For both years, three lines were found to have improved yield ($P < 0.1$), indicating that transgenic expression of ZmNF-YB2 in maize enhances yield under drought stress. Furthermore, when analyzed across lines within the construct, average yield was greater than the average of negative segregants. It is noteworthy that a greater yield benefit, for two of the three lines, was observed during 2003, under a more intense drought stress as compared with 2004. Indeed, under such relatively severe conditions, the best-performing transgenic maize line (Line 3) produced an ≈50% increase in yield relative to controls in the same experiment.
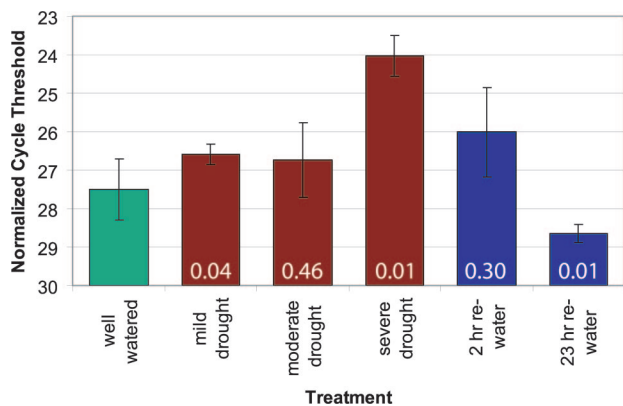

Fig. 4. Quantitative RT-PCR was used to measure *AtNF-YB1* transcript accumulation in wild-type plants over a drought timecourse. Relative water contents for the indicated treatments were 0.82 (well watered), 0.81 (mild drought), 0.60 (moderate drought), 0.29 (severe drought), 0.65 (2 h rewater), and 0.81 (23 h rewater). The *y* axis indicates the 18S RNA-normalized PCR cycle threshold for transcript detection. At severe drought treatment, *AtNF-YB1* crosses the detection threshold ≈3.5 cycles before the transcript in well watered conditions, indicating an ≈12-fold increase in expression during severe drought. Error bars indicate the standard deviation measured in two biological replicates. Numbers at the base of each bar indicate the *P* value resulting from a heteroscedastic two-tailed *t* test between the well watered and each drought treatment.

Fig. 5. Transgenic maize plants in greenhouse and field have visually observable improved drought tolerance. In both photographs, controls are in the left flat or row, and transgenics expressing ZmNF-YB2 are in the right flat or row.

Discussion

We have discovered that expression of certain CCAAT-binding transcription factors in plants imparts significant tolerance to drought, resulting in increased yield. This represents a previously unrecognized function for the NF-Y gene family in plants. To date, knowledge of the function of plant NF-Y genes has been limited. Recently, a number of NF-YB subunits in rice have been inferred as necessary for chloroplast function based on data from antisense and RNAi experiments (22). More recently, representatives of the NF-YC and NF-YB subunits of the trimeric complex have been implicated in the recruitment of CONSTANS-Like transcription factors to their DNA targets *in planta*, potentially mediating the effect of CONSTANS-like proteins on flowering time (23, 24). Additionally, in *Arabidopsis*, the LEC1 and LEC1-like proteins, which encode NF-YB subunits, have been found to be essential for embryo development. The LEC1 and LEC1-like proteins are phylogenetically and functionally distinct from other NF-YB subunits such as *AtNF-YB1* (refs. 25 and 26; SI Fig. 8). Indeed, it seems likely that a number of the other non-LEC1-like NF-YB subunits have related or overlapping roles to *AtNF-YB1*. This is supported by the fact that we have been unable to detect any marked changes in drought sensitivity in either of two independent T-DNA insertion lines for *AtNF-YB1* (data not shown).

Drought tolerance is a novel function for the NF-Y transcription factor family that presumably evolved through diversification among members of the gene family encoding the B subunit. Despite the complexity of overcoming drought-induced effects

Table 2. Drought-related phenotypes impacted by ZmNF-YB2

Phenotype	Genotype	Mean	Difference	SE	P
Chlorophyll, SPAD index	Transgenic	42.4	2.9	0.4	<0.001
	Control	39.4			
Leaf temperature, °C	Transgenic	38.2	−0.3	0.1	0.003
	Control	38.5			
Photosynthesis, μmol CO_2 m^{-2}·s^{-1}	Transgenic	18.7	3.4	1.3	0.010
	Control	15.3			
Stomatal conductance, mmol H_2O m^{-2}·s^{-1}	Transgenic	123	24	8	0.011
	Control	99			

in land plants, modulated expression of individual signal transduction pathways has emerged as a good strategy for conditioning enhanced drought tolerance in plants. The same strategy is expected to be applicable to many crops commonly grown in locations subject to drought. These crop results have validated our functional genomics approach in the *Arabidopsis* model for identifying novel drought tolerance mechanisms with practical applications in crop production.

Our data support a role for the *AtNF-YB1* and the *ZmNF-YB2* genes in a drought-adaptive mechanism. This adaptive mechanism is regulated by a set of structurally conserved CCAAT family members conserved in both dicots and monocots. The observed drought-tolerance mechanisms elicited through constitutive expression of NF-YB proteins are components of normal responses to drought conditions. The stress-adaptation responses contribute to a yield advantage in maize that is grown within drought environments. The application of this technology is therefore expected to have the most significant impact on severely water-limited maize production systems. A focus of our ongoing work is to characterize the effects of this technology over a greater range of environments. Further technical refinements, such as more specifically controlled expression, may be needed to expand the application to environments that experience less-severe or sporadic drought effects.

Materials and Methods

***Arabidopsis* Cloning, Transformation, and Mutant Line Generation.** Experiments were performed by using *Arabidopsis* Col-0. The *AtNF-YB1* cDNA (T45165) was obtained from a library generated by the MSU-DOE Plant Research Laboratory at Michigan State University (East Lansing, MI). The clone was resequenced and
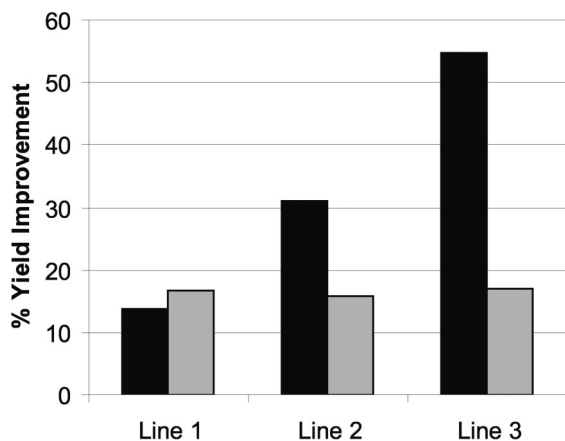
Fig. 6. Three transgenic maize lines demonstrate improved yield in 2 years of yield testing. Values plotted are increase on a percentage basis of transgenics over controls. All differences plotted are significant at *P* < 0.1. Data from three independent lines are shown with side-by-side comparison of 2 years' results. Base yield (yield of controls) was 4.6 metric tons/hectare (74 bushels/acre) in Year 1 and 6.4 metric tons/hectare (102 bushels/acre) in Year 2.

deposited in the GenBank database (accession no. DQ333305). *Arabidopsis* plants were transformed by the floral dip method (27, 28) using *Agrobacterium* carrying a standard transformation construct P46, which contained a kanamycin-resistance selectable marker system driven by a nopaline synthase promoter and the *AtNF-YB1* cDNA downstream from the cauliflower mosaic virus 35S promoter. Homozygous mutants for each of two independent T-DNA insertion alleles within *AtNF-YB1* were established from seed lots supplied by the *Arabidopsis* Biological Research Center at Ohio State University (Columbus, OH). Both insertions were derived from the SALK collection (SALK_032272.55.75.x, GenBank accession no. BH612182.1, and SALK_109993.42.55.x, GenBank accession no. BZ664699.1). Homozygotes for each of these insertions showed wild-type morphology except for a slight acceleration in flowering. Constitutive expression of *AtNF-YB1* in the 35S::*AtNF-YB1* lines was verified by RT-PCR experiments on RNA extracted from shoots and roots. Constitutive expression of *AtNF-YB1* protein in the 35S lines and its absence in the mutant lines was confirmed by Western blots using an *AtNF-YB1* antibody.

***Arabidopsis* Drought-Survival Assays.** Seeds were sterilized by a 2-min EtOH treatment followed by 20 min in 30% bleach/0.01% Tween and five washes in distilled water. Seeds were sown on MS agar in 0.1% agarose and stratified for 3 days at 4°C before transfer to growth chambers with a temperature of 22°C. After 7 days of growth on plates, seedlings were transplanted to 9-cm-diameter clay pots containing a 1:1 vermiculite:perlite mixture topped with soil. Each pot contained 14 seedlings of a transgenic line or the control plants, and 5–45 pots each of a transgenic line and the control were included in each run of the experiment. Pots were placed in a growth room under 24-h light conditions (18–23°C and 90–100 μmol photosynthetic active radiation m^{-2}·s^{-1}) and watered for a period of 14 days. To apply a drought treatment, water was then withheld, and pots were placed on absorbent paper for a period of 8 days. After 8 days of water deprivation, pots were rewatered. The number of surviving plants in each pot was counted at ≈5 days after rewatering.

***Arabidopsis* Drought Physiology Experiments.** Five-week-old *Arabidopsis* plants grown under a 10-h day with an average daytime temperature of 21°C were used in this experiment. Plants were grown in 10-cm pots. To impose drought, water was withheld for 8 days followed by a partial recovery adding 20 ml of water per pot. The drought was extended for 3 more days, followed by a full rewatering and recovery. Physiological measurements were made on plants during drought when severely stressed. Assimilation of CO_2 was estimated by using a LICOR-6400 CO_2 gas exchange analyzer fitted with a LICOR 6400–15 *Arabidopsis* leaf chamber (LICOR, Lincoln, NE) and measuring the largest most fully mature leaf. To attempt to distinguish stomatal from nonstomatal effects of drought on photosynthesis, a saturating CO_2 level of 1,000 ppm was provided by using the 6400-01 CO_2 injection system and CO_2 cartridges using a flow of 500 μmol s^{-1}. Measurements were taken when the coefficient of variation over the previous 15 s for changes

in CO_2, water, and flow was <1%. A metal-halide lamp cooled with a water bath provided light at a field-relevant photosynthesis-saturating level of 1,000 μmol photosynthetic active radiation m^{-2}·s^{-1}. *Arabidopsis* leaf water potential was measured by thermocouple psychrometry with a 14-channel Wescor/Campbell Water Potential System [C-52 sample chambers (WESCOR, Logan, UT), plus the CR 7 Measurement and Control System (Campbell, Logan, UT)]. Leaf discs from the youngest fully expanded leaf were placed in the sample chamber. Data were collected and converted to water potential with the CR7 Measurement and Control System and the company-provided software.

Corn Cloning, Transformation, and Expression Analysis. Bioinformatic analysis was used to identify maize homologs of *AtNF-YB1*. The corn NF-YB gene tested here (*ZmNF-YB2*) was obtained from a nmh56′//′]Monsanto maize cDNA library; its sequence has been deposited under GenBank accession no. DQ333304. A corn transformation vector was prepared with rice actin promoter and 5′ UTR with an embedded intron (OsRACT) driving the expression of *ZmNF-YB2*. The selectable marker cassette for this vector included *npt* II driven with an enhanced cauliflower mosaic virus promoter and *Agrobacterium* nopaline synthase terminator. Transformation of an elite maize inbred was by the method described by Armstrong and Rout (29). Maize transgenic lines with either one or two copies of the construct and no oriV origin of replication from the vector were selected. All transgenic lines were confirmed to express the transgene in multiple subsequent generations via an anti-NF-YB antibody.

Corn Greenhouse Drought Experiments. Pregerminated seedlings of transgenic plants (progeny of a heterozygous transgenic plant that inherited the exogenous transcription factor DNA construct) and wild-type plants (progeny of a heterozygous transgenic plant that did not inherit the exogenous transcription factor DNA construct) were planted in 12.5-cm pots containing 330 g of soil. The plants were well watered for 1 week and then allowed to dry for 4 days. An equal number (32) of transgenic and wild-type plants were selected based on matched height, and the selected plants were mixed in eight flats. Four flats were designated as "wet," meaning they would be well watered, and four flats were designated as "dry," meaning they would be subjected to drought. All pots were brought to the weight of the heaviest pot by adding water, with well watered pots weighing ≈800 g. The pots were weighed daily until the average pot weight dropped to between 600 and 700 g. At that weight, a drought assay was started by measuring plant heights and resuming watering for pots in wet flats while continuing to withhold water for pots in dry flats. The pots in the wet flats were fully watered daily. The pots in the dry flats were weighed daily to determine a drought treatment. If the average dry flat pot weight was >500 g, no water was added; if the average pot weight was between 365 and 500 g, 35 g of water was added to each pot; if the average pot weight was <365 g, a determined amount of water was added to bring the average pot weight to 400 g. The drought treatment was continued until the pots in the dry flats had an average pot weight <500 g for 8 days. The height of all transgenic and control plants was measured on the ninth day. On the ninth day, full watering was resumed for the dry flat pots for 3 days, after which heights were again measured. Recovered plants were subjected to a second round of drought as described above. After 9 days of drought, full water was resumed for 7 days.

Corn Field Physiology Experiments and Yield Testing. The chlorophyll index was measured by using a Minolta (Spectrum Technologies, Plainfield, IL) SPAD 502 Meter. Photosynthesis, stomatal conductance, and leaf temperature were measured by using the PP Systems (Amesbury, MA) Ciras-1 Portable Photosynthesis System. Leaf photosynthesis was measured at an atmospheric $[CO_2]$ of 367 μmol·mol^{-1}, an ambient water vapor pressure of 2.3 kPa, and a leaf air vapor pressure deficit between 0.6 and 1.5 kPa. Measurements were made midday with photosynthetic photon flux density between 1,200 and 1,400 μmol·m^{-2}·s^{-1}, attained by orientating the leaf chamber perpendicular to incident light. Measurements were made midway along the most recent fully expanded leaves and measured on six plants within each of six field replications, per event and per control, during the drought period. Yield data were collected from two experiments. In the first year, data were evaluated from a single six-replication drought efficacy trial using a Split-Split-Split block design in Kansas. This trial was the source of the physiology data shown. In the following field season, a multilocation study using group unbalanced block design with three replicates within each location was performed. All inbred lines were tested as two different maize hybrids, and four commercial varieties were planted as additional controls. For all drought trials, supplied irrigation kept plants well watered at all periods except during flowering, when irrigation was withheld to achieve an ≈7.5-cm water deficit compared with normal irrigation practice. Multiple independent lines were included in the tests to reduce the potential influence of positional effects on expression levels or the activity of genes neighboring the insertion site that in turn might impact efficacy and yield.

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