Journal of Genetic Engineering and Biotechnology 22 (2024) 100352

Contents lists available at ScienceDirect

Journal of Genetic Engineering and Biotechnology

journal homepage: www.elsevier.com/locate/jgeb

Efficacy of Event MON 87460 in drought-tolerant maize hybrids under optimal and managed drought-stress in eastern and southern africa

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ARTICLE INFO

Keywords:
CspB gene
DroughtGard®
DroughtTEGO® hybrids
Gene efficacy
Managed drought-stress
MON 87460 Event
Optimum-moisture
Traited transgenic hybrids
Yield penalty
Zea mays

ABSTRACT

Background: Frequent drought events due to climate change have become a major threat to maize (*Zea mays* L.) production and food security in Africa. Genetic engineering is one of the ways of improving drought tolerance through gene introgression to reduce the impact of drought stress in maize production. This study aimed to evaluate the efficacy of Event MON 87460 (*CspB*; *DroughtGard*®) gene in more than 120 conventional drought-tolerant maize hybrids in Kenya, South Africa, and Uganda for 3–6 years under managed drought-stress and optimal conditions and establish any additional yield contribution or yield penalties of the gene in traited hybrids relative to their non-traited isohybrids. Germplasm used in the study were either MON 87460 traited un-adapted (2008–2010), adapted traited *DroughtTEGO*® (2011–2013) or a mix of both under confined field trials.

Results: Results showed significant yield differences ($p < 0.001$) among MON 87460 traited and non-traited hybrids across well-watered and managed drought-stress treatments. The gene had positive and significant effect on yield by 36–62% in three hybrids (CML312/CML445; WMA8101/CML445; and CML312/S0125Z) relative to non-traited hybrids under drought, and without significant yield penalty under optimum-moisture conditions in Lutzville, South Africa. Five traited hybrids (WMA2003/WMB4401; CML442/WMB4401; CML489/WMB4401; CML511/CML445; and CML395/WMB4401) had 7–13% significantly higher yield than the non-traited isohybrids out of 34 adapted DroughtTEGO® hybrids with same background genetics in the three countries for ≥ 3 years. The positive effect of MON 87460 was mostly observed under high drought-stress relative to low, moderate, or severe stress levels.

Conclusion: This study showed that MON 87460 transgenic drought tolerant maize hybrids could effectively tolerate drought and shield farmers against severe yield loss due to drought stress. The study signified that development and adoption of transgenic drought tolerant maize hybrids can cushion against farm yield losses due to drought stress as part of an integrated approach in adaptation to climate change effects.

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https://doi.org/10.1016/j.jgeb.2024.100352
Received 12 December 2023; Received in revised form 14 January 2024; Accepted 15 January 2024

1. Background

Maize (*Zea mays* L.) is a major cereal crop that accounts for the highest production areas and volumes globally[19]. Maize grain is mainly used as human food, animal feed, and raw materials for various industrial products[57,58,64]. Maize importance has increased due its use beyond food and feed as biofuel and biotech crop[55,66]. In sub-Sahara Africa (SSA) the larger population depends heavily on maize as a staple and the increasing population means increased demand for maize grain. Therefore, global maize production must be increased in tandem with the demand, while pressure on agricultural land constrains expanding production area[24]. Production must, therefore, be maximized on the limited arable land.

Besides limited land for production, various biotic and abiotic stresses are a major constraint to maize production and grain quality[23,26]. Globally, drought, waterlogging, and extreme temperatures have negatively affected maize production[3,67]. These challenges have been exacerbated by climate change. Drought is particularly a critical factor contributing to significant maize yield losses[26,60,24]. Maize yield losses vary considerably from 30 to 90% depending on the severity of stress and the effect is more during flowering and grain filling stages[51]. Breeding for drought stress tolerance is, therefore, important to develop resilient crop varieties that yield better in stress environments[8,26].

The development of drought tolerant varieties can be achieved through both conventional and biotechnology breeding approaches[14,30]. Most conventionally bred maize hybrids currently on the market in Africa have been developed to exhibit some degree of drought tolerance. These are products from some major interventions to drought viz Drought Tolerant Maize for Africa (DTMA), Stress Tolerant Maize for Africa(STMA) and Water Efficient Maize for Africa (WEMA) have been undertaken to develop drought tolerant maize hybrids that were widely evaluated on-farm for adaptation and are being grown by farmers in various countries[20,43,15,7]. Other breeding programs have evaluated germplasm for identification of drought tolerance trait sources in Algerian maize populations for utilization in conventional breeding[10,48,33,50,4,49,47].

Although advances in conventional plant breeding have made significant contributions to improving maize for drought tolerance, the process is cumbersome in identifying sources of the trait, incorporation of the trait in the target germplasm, and development of drought tolerant varieties due to the complex nature of the trait; and how it is controlled by multiple genes and signalling pathways[9,22]. Drought trait expression is also influenced genotype × environment [G × E] interaction and exhibits low yield heritability effects[68,34].

In recent times, molecular breeding, genome-wide marker-assisted selection, including transgenic approaches and gene-editing technologies are additional tools for drought tolerance trait improvement[30,56]. Molecular markers have transformed and improved the efficiency of breeding drought tolerant crops in a major way[5,39]. However, molecular breeding research has limitations and has not progressed beyond the detection of a specific trait (QTLs) under drought stressed condition while MAS contribution to the development of drought-tolerant varieties has not been widely applied and reported in literature[56,7].

Despite all the efforts in conventional and molecular breeding for drought stress breeding, drought stress is still a constraint in maize production, mostly due to unpredictable weather events from climate change becoming more frequent, and crop adaptation will require better strategies[59,56].

Drought tolerance traits introduced through biotechnology into conventionally bred drought-tolerant germplasm could further improve maize yield stability under moisture stress[38,66,30,56]. Biotechnology breeding approach has the advantage of taking shorter breeding time, uses major genes and therefore, saves resources and provides opportunities to address production constraints in a timely manner[16,29,2]. Transgenic technology is one of the techniques used to improve drought tolerance in crops. Genetic engineering provides more options to quickly improve drought tolerance by incorporating natural and synthetic genes and transcription factors at low costs into maize germplasm to enhance drought tolerance[45,35]. Genetic engineering of maize with desired target genes has been extensively employed to produce transgenic maize varieties with improved drought stress tolerance. The first transgenic maize varieties were launched commercially in 1996 in the USA and maize has since received a lot of attention in plant genetic engineering[38,30]. One such trait used to enhance drought tolerance in maize is Event MON 87460 (also known as *DroughtGard®)*, a cold shock protein B (C*spB*) from *Bacillus subtilis*[27,52,54].

MON 87460 transgenic maize hybrids showed significant yield increase under water-stress conditions in the USA compared with non-transgenic hybrids[37,61] and MON 87460 traited maize was the first abiotic stress-tolerant crop plant developed through biotechnology to achieve commercial introduction in USA in 2013/2014[28]. Both conventional and transgenic drought-tolerant hybrids are widely adopted in the USA[36]. Proof of concept studies in the USA done over a period of four years indicated that *CspB* (MON 87460) traited hybrids provided an average yield increase of 10.5%[12]. It is, therefore, hypothesized that the incorporation of *CspB* gene into conventionally developed climate-resilient *DroughtTEGO®* hybrids through the Water Efficient Maize for Africa (WEMA) Project[44,11,17,43,46] could further enhance the level of tolerance with an additional yield increase of 8–10%.

However, like conventional maize, MON 87460 traited maize is still subject to yield loss under water-limited conditions. The impacts of drought stress on yield loss could be less severe under moderate drought-stress. Under severe water deficit, both MON 87460 and conventional maize hybrids could suffer complete yield loss[31,32].

The objectives of this study were to: (a) Evaluate the efficacy of Event MON 87460 drought tolerance (DT) transgenic trait in confined field trials (CFTs) in Kenya, Uganda, and South Africa under managed drought-stress and optimal conditions; (b) Establish DT transgene additional yield contribution in converted Event MON 87460 hybrids; and (c) Identify any yield penalties in traited hybrids relative to their isohybrids under managed drought-stress and optimal conditions.

2. Methods

2.1. Evaluation sites

Five sites used for the evaluation of the efficacy of Event MON 87460, germplasm types, and planting and harvesting dates are described in Table 1. The sites are low lying areas with very little and unreliable rainfall, but ideal for managed drought stress crop trialing. The evaluations were done as confined field trials (CFTs), meaning that the trials were carried out under biosafety regulatory confinement, with restricted access and isolation from other maize crops to prevent gene flow through cross-pollination from the trial into neighboring maize fields; and to prevent materials from entering the food chain before receiving biosafety approval for open cultivation from the relevant national biosafety regulatory authorities.

2.2. Germplasm

Germplasm used in the trials included Event MON 87460 (*CspB*) traited DT-hybrids and their corresponding non-transgenic hybrid versions (isogenic hybrids – isohybrids) sourced from Monsanto Company (now Bayer) and CIMMYT. Commercial non-transgenic maize varieties were used as controls (checks). Most of the traited germplasm are

Table 1

Description of the five sites, and planting and harvesting dates for evaluation of efficacy of DT MON 87460 in Kenya, South Africa, and Uganda from 2008 to 2013.

Country	Locations	Altitude (m asl)	Latitude		Yr Planted	Yr Harvested
South Africa	Hopetown*	1111	29.53858° S	23.9363° E	2007	2008
	Orania*	1180	29.7831^0 S	24.44677^0 E	2007	2008
South Africa	Hopetown*	1111	29.53858° S	23.9363° E	2008	2009
	Orania*	1180	29.7831^{0}S	24.44677^0 E	2008	2009
South Africa	Hopetown *	1111	29.53858° S	23.9363° E	2009	2010
	Orania*	1180	29.7831^{0}S	24.44677^0 E	2009	2010
	Lutzville*	2	31.5924^O S	18.3781^{o}E	2009	2010
South Africa	Hopetown;	1111	29.53858° S	23.9363° E	2010	2011
	Orania,	1180	29.7831^0 S	24.44677^0 E	2010	2011
	Lutzville*	2	31.5924^O S	18.3781^{o}E	2010	2011
South Africa	Hopetown***	1111	29.53858° S	23.9363° E	2011	2012
	Orania***	1180	29.7831^{0}S	24.44677^0 E	2011	2012
	Lutzville ***	2	31.5924^O S	18.3781^{o}E	2011	2012
South Africa	Hopetown ***	1111	29.53858° S	23.9363° E	2012	2013
	Orania***	1180	29.7831^{0}S	24.44677^0 E	2012	2013
	Lutzville***	2	31.5924^O S	18.3781^{o}E	2012	2013
Kenya	Kiboko**	929	2.2103° S	37.7231° E	2010	2011
Kenya	Kiboko**	929	2.2103° S	37.7231° E	2011	2012
Kenya	Kiboko**	929	2.2103° S	37.7231° E	2012	2013
Kenya	Kiboko**	929	2.2103° S	37.7231° E	2013	2014
Uganda	Kasese**	1007	0.1699° N	30.0781° E	2010	2011
Uganda	Kasese**	1007	0.1699° N	30.0781° E	2011	2012
Uganda	Kasese**	1007	0.1699° N	30.0781° E	2012	2013
Uganda	Kasese**	1007	0.1699° N	30.0781° E	2013	2014

*Un-adapted germplasm planted, ** adapted germplasm planted, *** mix of un-adapted and adapted germplasm planted.

parents of the commercially released, conventionally bred climate-smart *DroughtTEGO*® hybrids from the WEMA Project[44,11,17,43,46]. Overall, more than 120 traited hybrids were evaluated and the efficacy of the DT Event MON 87460 compared with non-traited isohybrids and commercial checks.

The efficacy study is done in confinement to provide maximum response that can be achieved with the DT gene as a proof-of-concept that the gene works in tropical African germplasm to permit the deregulation of the DT gene by the national biosafety regulatory authorities for open cultivation by farmers. After the DT gene deregulation, the traited hybrids with the DT gene are further evaluated for their yield performance and stability across the maize growing agroecologies before they are approved for cultivation by farmers.

Germplasm evolution was such that in years 1–3 of the CFTs, un-adapted Bayer germplasm was used. Later, in Years 4–6, adapted germplasm from Bayer and CIMMYT, comprising Bayer lines × Bayer lines; Bayer lines × CIMMYT lines; and CIMMYT lines × CIMMYT lines) were evaluated in the CFTs. In these crosses, the DT gene was always in the male parent. Four commercial checks were used in the trials in Kenya (DKC80-53, DK8031, PHB3253, and WH505); four in Uganda (Longe-10H, Longe-6H, DH04, and PAN67); and two (PHB32D99 and DKC73-76R) in South Africa.

2.3. Trial treatments and experimental design

Event MON 87460 traited hybrids were evaluated in CFTs under optimum-moisture and managed drought-stress regimes in Kenya and Uganda. In South Africa, the materials were tested under three targeted treatments: Well-watered (optimum), flowering stress (moderate) and chronic (severe) drought stresses. The stress levels were later separated into individual levels after harvest when the actual yield reductions under drought stress relative to optimum condition were established: “Stress” = average of all different stress levels; “Severe” = >75% yield reduction; “High” = 50–75% yield reduction; “Moderate” = 25–50% yield reduction; and “Low” = <25% yield reduction compared with optimum-moisture condition. The trials were planted in drought prone environments during rain-free windows at all sites to allow for drought stress management. Drip irrigation was installed at all the sites to provide the required water during the vegetative growth stage. Under drought stress trials, supply of water was stopped at 2–3 weeks before flowering until 2–3 weeks after flowering; and irrigation of the trials was resumed thereafter.

In South Africa, a two-factor group block experimental trial design was used, with well-watered (optimum) and water stress as main treatments. The entries were planted in 20 replications for managed drought-stress trials and in six replications for optimum trials. In Kenya and Uganda, the trials were planted in an alpha-lattice experimental design in two blocks, with four replications. Each entry was planted in a 2-row, 5-m long plot at 75 × 25 cm spacing. Two seeds were planted per hill and thinned to one seedling per hill at two weeks after emergence. This made a total of 21 plants per row with a target population of 53,333 plants ha^{-1}. Standard rates of fertilizers were applied as basal fertilizer (125 kg K_2O and 125 kg P_2O_5 ha^{-1}) at planting; and top-dressed with urea at 120 kg N ha^{-1}. The fields were kept weed-free by spraying appropriate herbicides and hand weeding; and sprayed with appropriate pesticides to protect against stemborer damage.

To determine grain yield, the focus of the study, ears from each plot were harvested from all the plants, weighed and their moisture content determined from a sample of grains. The yield was then converted to grain yield per hectare, assuming 80% shelling percentage and adjusted for moisture content at 13.5% using the formula below:

Dry grain weight (kg/ha) = [field weight (kg) × (1-Field MC)/0.865)]/plot area (ha).

Where MC = Moisture content.

3. Data analysis

The means of the grain yield were calculated for each experimental unit in Microsoft Excel. The statistical analyses for this study were done using PROCMIXED in SAS[53]. Summary statistics were generated on mean yields, mean Delta, $LSD_{(0.05)}$, Delta % and *P*-values. The means were compared using multiple comparison tests of Fisher's LSD method. Pairwise comparison of traited vs. non-traited was done using *t*-test.

Pairwise comparison was used to summarize the performance of traited MON 87460 hybrids against non-traited hybrids to show yield differences (Delta – t/ha and % Delta). Percentage yield differences between traited and non-traited versions of the hybrids with same base genetics, tested across years, countries, and locations were compared to show the yield increase due to the DT gene as a proof-of-concept that the gene works in tropical African germplasm.

The level of statistical significance for comparisons was predetermined at $P = 0.05$. Since some hybrids were evaluated more times than others, the weighted contribution of each hybrid was carried out based on the number of data points to show the number and magnitude of the bars having a positive effect versus those having a negative effect (penalty) on grain yield.

4. Results

4.1. Overall efficacy of MON 87460 in traited hybrids evaluated in East Africa and South Africa

Combined analysis of variance was conducted for grain yield, comparing traited and non-traited hybrids across six years in South Africa; and across three years in both Kenya and Uganda, and comparing means across well-watered and stressed treatments in each location (Table 2). Results showed that on average, traited hybrids yielded significantly higher than non-traited hybrids ($p < 0.001$) (Table 2).

Under optimum-moisture (well-watered) conditions, there were no significant differences between traited and non-traited hybrids, as required for biosafety regulatory trials, thus, confirming that there were no unintended changes due to genetic transformation using the DT gene. Under stressed conditions, the hybrids with gene yielded significantly more than those without the gene (Table 2). Performance under different stress levels showed that the traited hybrids yielded significantly higher than the non-traited hybrids by 4 to 7.7% under severe- and high-stressed conditions. However, there was no significant yield difference between traited and non-traited hybrids under moderate- and low-stress levels (Table 2).

4.2. Efficacy of MON 87460 in traited hybrids evaluated in South Africa, across years and locations

Overall, across well-watered and stressed treatments and under optimum-moisture conditions, traited hybrids yielded significantly higher ($p < 0.01$) than non-traited hybrids across locations in South Africa (Table 3). Under severe and high stress conditions, the traited hybrids yielded significantly ($P < 0.001$) higher than the non-traited hybrids by 4.5 to 8.8%, while under moderate and low stress levels, there was no significant yield difference between traited and non-traited hybrids (Table 3).

4.3. Efficacy of MON 87460 in traited hybrids evaluated in East Africa, across years and locations

A summary of all yield data collected in East Africa (Kenya and Uganda), comparing traited non-traited hybrids (Fig. 1a) averaged across well-watered and stressed treatments, showed there was no significant difference in yield between traited hybrids and non-traited hybrids. Under optimum-moisture conditions, there was significant ($P < 0.05$) yield increase in about 2% of the non-traited hybrids compared with the traited hybrids (Fig. 1a). Under severe and moderate stress conditions, there were no significant differences in yield between the traited and non-traited hybrids. However, the traited hybrids yielded 3.4% higher ($P < 0.05$) under high stress condition than the non-traited hybrids (Fig. 1b).

4.4. Efficacy of MON 87460 in traited hybrids tested in managed stress environments in Kenya, Uganda, and South Africa

Percentage yield differences between traited and non-traited versions of the 120 hybrids with the same background genetics, tested across six years, three countries and five locations showed a positive MON 87460 gene effect in about 50% of the hybrids tested in Kenya, Uganda, and South Africa under drought stress (Fig. 2). The top five MON 87460 traited hybrids had significant yield advantages of 14 to 19% relative to the non-traited versions, while two traited hybrids had significant yield penalties (disadvantages) of 12–17% over the non-traited, isohybrids (Fig. 2). Weighted contribution of the hybrids showed that out of the 120 hybrids evaluated in Kenya, Uganda, and South Africa, about 50% showed a positive gene effect – yield increase (Fig. 3).

4.5. Efficacy of MON 87460 in 34 WEMA hybrids tested for over three years in managed drought stress environments in South Africa, Kenya, and Uganda

Performance of 34 hybrids with same background genetics initially developed as conventional drought tolerant hybrids branded as *DroughtTEGO®*, showed that 71% of the hybrids had positive gene effect on grain yield (Figs. 4a and 4b). Five traited single-cross hybrids – WMA2003/WMB4401; CML442/WMB4401; CML489/WMB4401; CML511/CML445; and CML395/WMB4401 had 7–13% (0.55–0.87 t ha^{-1}) greater ($P < 0.05$) yield than the non-traited versions of the hybrids (Figs. 4a and 4b).

4.5.1. MON 87460 efficacy in Kenya

In Kenya, there was no significant difference in yield reduction for traited, non-traited and check hybrids due to drought stress relative to optimum-moisture condition (Fig. 5). The level of yield reduction of 38–40% under drought is classified as “moderate stress”. Pairwise comparison showed that no hybrid had significant yield penalty while

Table 2

Pairwise comparison of yield of DT MON 87460 traited and non-traited hybrids under optimum-moisture and different drought stress levels across three countries (South Africa, Kenya, and Uganda).

Treatment	Traited N[1]	Non-traited N[1]	Traited Mean (t/ha)	Non-traited Mean (t/ha)	Delta (t/ha)	Delta (%)	Sign	$LSD_{0.05}$	SE
Overall	9305	9148	8.20	8.09	0.11	1.31	***	0.05	0.33
Optimum	2646	2615	11.20	11.11	0.09	0.83	NS	0.09	0.43
Stress	6659	6533	6.20	6.09	0.11	1.84	***	0.05	0.26
Severe stress	347	348	4.01	3.72	0.29	7.70	***.	0.17	0.67
High stress	2562	2463	4.85	4.65	0.20	4.28	***	0.06	0.30
Moderate stress	2331	2287	6.47	6.43	0.04	0.66	NS	–	–
Low stress	1318	1336	8.99	8.96	0.04	0.41	NS	–	–

[1] Number of data points used for statistical analyses; *** = $P < 0.001$; NS = Not significant at $P < 0.05$; SE = Standard error.

Table 3
Pairwise comparison of yield for MON 87460 traited and non-traited hybrids under optimum-moisture and different drought stress levels across three locations in South Africa.

Treatment	Traited N[1]	Non-traited N[1]	Traited Mean (t/ha)	Non-traited Mean (t/ha)	Delta (t/ha)	Delta (%)	Significance
Overall	7787	7640	8.47	8.34	0.12	1.65	***
Optimum	2124	2098	11.89	11.74	0.15	1.29	**
Stress	5663	5542	6.39	6.26	0.13	2.11	***
Severe stress	277	279	04.76	4.38	0.38	8.77	***
High Stress	2109	2008	4.97	4.76	0.21	4.51	***
Moderate stress	1858	1820	6.56	6.47	0.08	1.28	NS
Low Stress	1318	1336	8.99	8.96	0.037	0.41	NS

[1] Number of data points used for statistical analyses; *** = $P < 0.001$; ** = $P < 0.01$; NS = Not significant at $P < 0.05$.

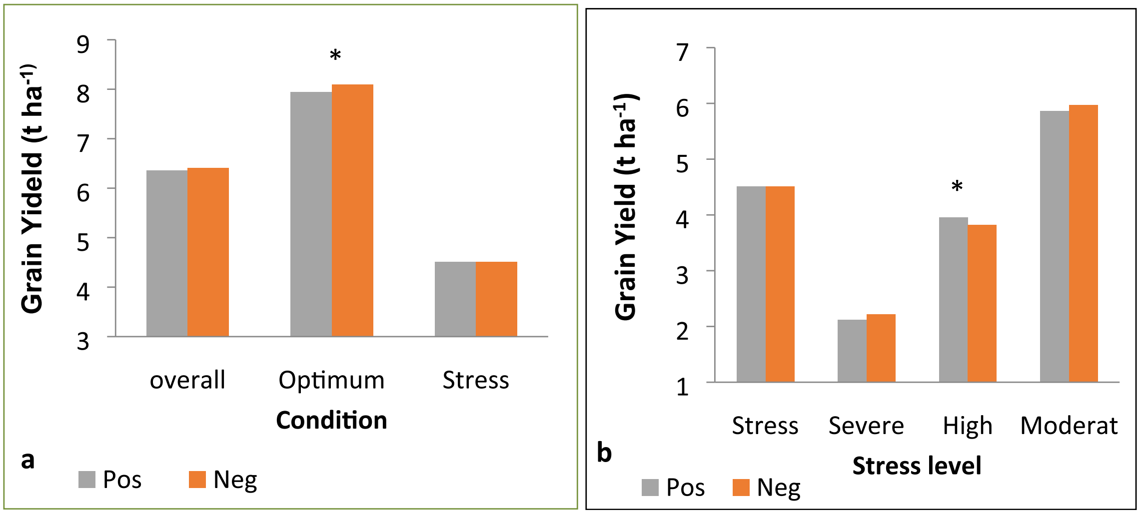

Fig. 1. 1a: Performance of MON 87460 for traited (pos) vs. non-traited (neg) hybrids under optimum-moisture and; 1b: performance of MON 87460 for traited (pos) vs. non-traited (neg) hybrids under different drought stress levels, in Kenya and Uganda across years, countries, and locations. (* Significantly different at $P < 0.05$ for traited vs. non-traited yield bars).

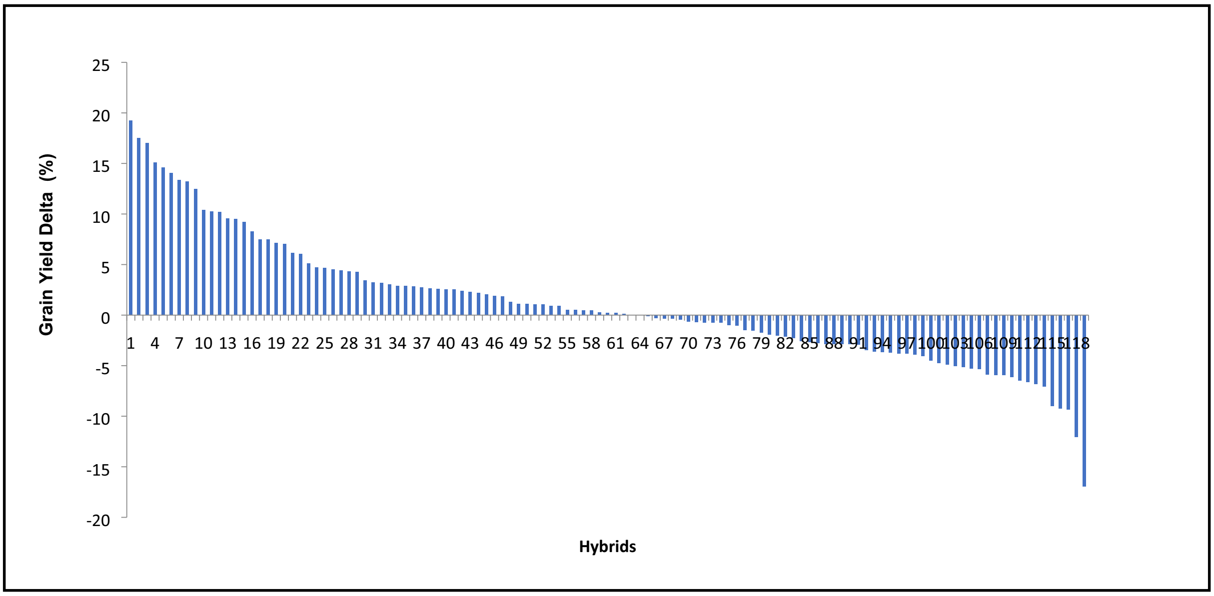

Fig. 2. Percentage (Delta %) yield difference between traited and non traited hybrids across managed drought-stress environments for six years in South Africa, Kenya, and Uganda.

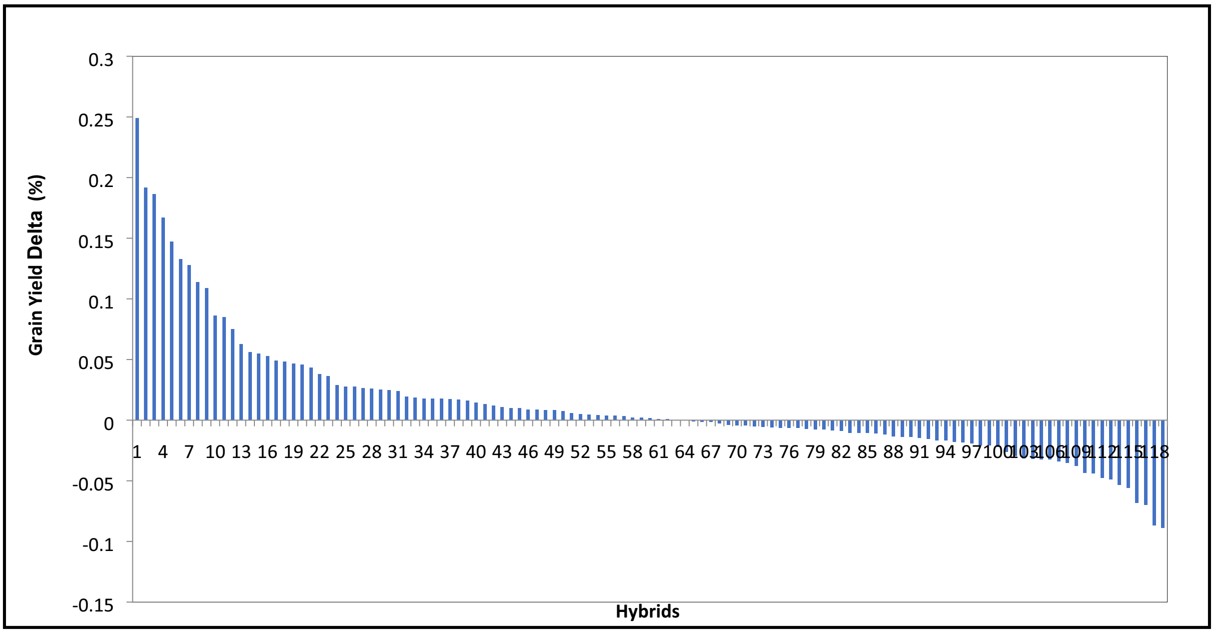

Fig. 3. Weighted percentage yield difference between traited and non traited hybrids under managed drought-stress environments in South Africa, Kenya, and Uganda.

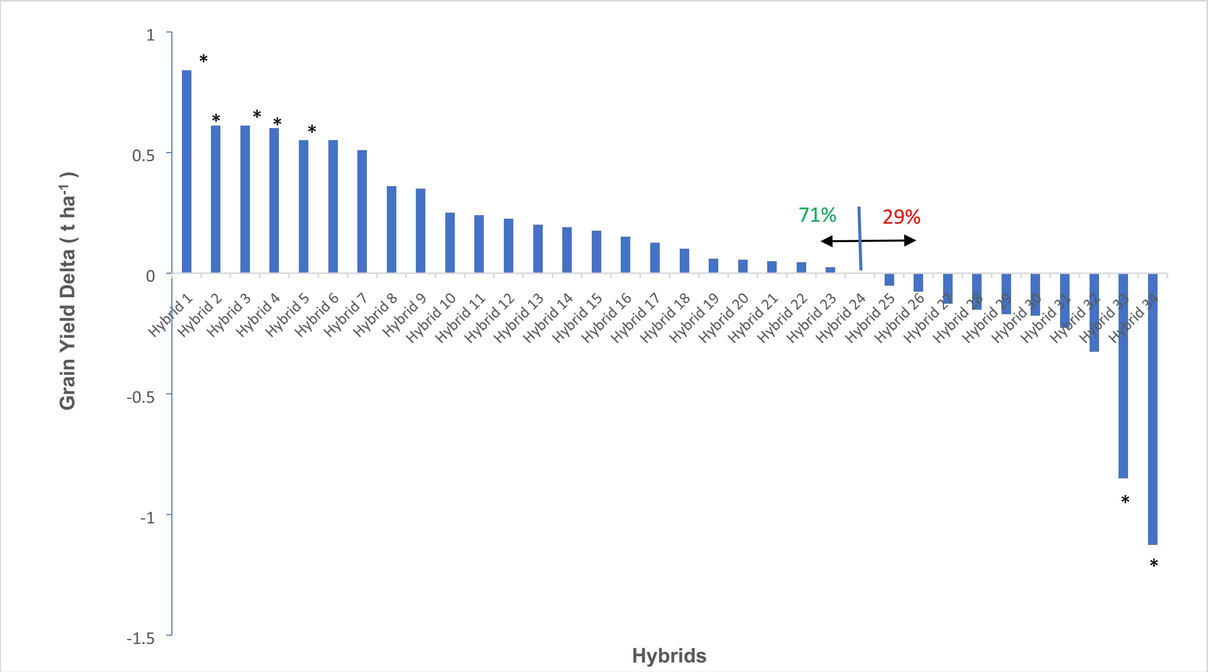

Fig. 4a. Delta (actual) yield difference between the traited hybrids (with the DT gene) and non-traited hybrid versions (without the DT gene) among 34 adapted hybrids with same genetics evaluated under managed drought-stress environments in South Africa, Kenya and Uganda. * = Significantly different at P < 0.05.

four hybrids had significantly more yield (15.8–29.2%) under optimum-moisture condition (Table 4).

Two traited hybrids had significantly more yield under moderate drought stress compared with non-traited versions. Differences between traited and non-traited hybrids ranged from 0.95 to −0.70 t ha^{-1} (21.1 to −11.7%) (Table 5). Two single-cross hybrids, WMA2003/WMB4401 and CML445/WMB4401, had a significant yield increase of 17–21% (0.62–0.94 t ha^{-1}) of the DT trait under moderate drought (Table 5). However, one hybrid (CML312/CML445) had significant yield penalty (-11.7%) under moderate drought.

4.5.2. MON 87460 efficacy in Uganda

Yield reductions of traited, non-traited, and check hybrids due to managed drought relative to optimum-moisture condition, were 65, 67 and 74%, respectively (Fig. 6); suggesting that the level of drought stress was “high”. Pairwise comparison showed that there were no significant yield differences between the traited and non-traited versions of the hybrids under optimum conditions. The differences between traited and non-traited hybrids ranged from 2.32 to −3.00 t ha^{-1} (28.5 to −21.4%) (data not shown).

Overall, top three hybrids had differences between traited and non-traited hybrids ranging from 0.47 to 0.80 t ha^{-1} (12.6–28.2%) com-

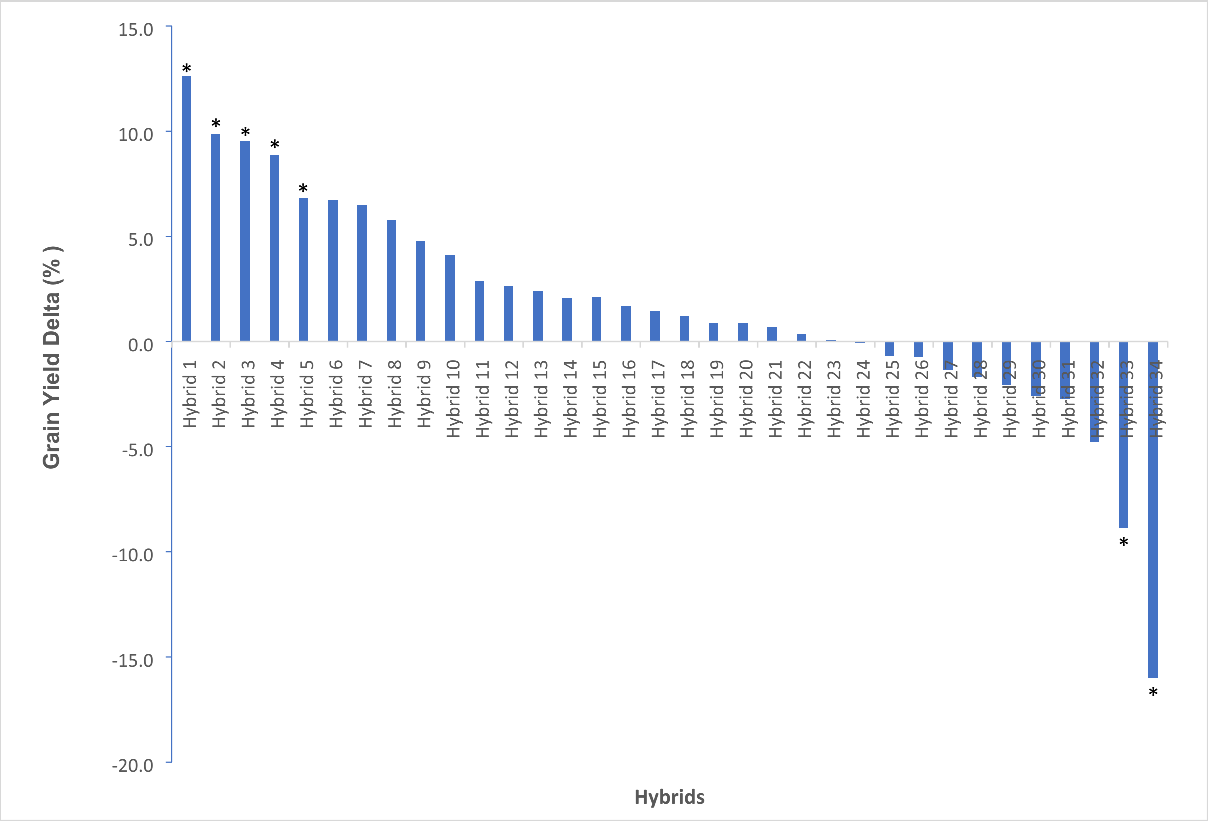

Fig. 4b. Percentage Delta yield (difference) between the traited hybrids (with the DT gene) and non-traited hybrid versions (without the DT gene) among 34 adapted hybrids with same genetics evaluated under managed drought-stress environments in South Africa, Kenya, and Uganda. * = Significantly different at $P < 0.05$.

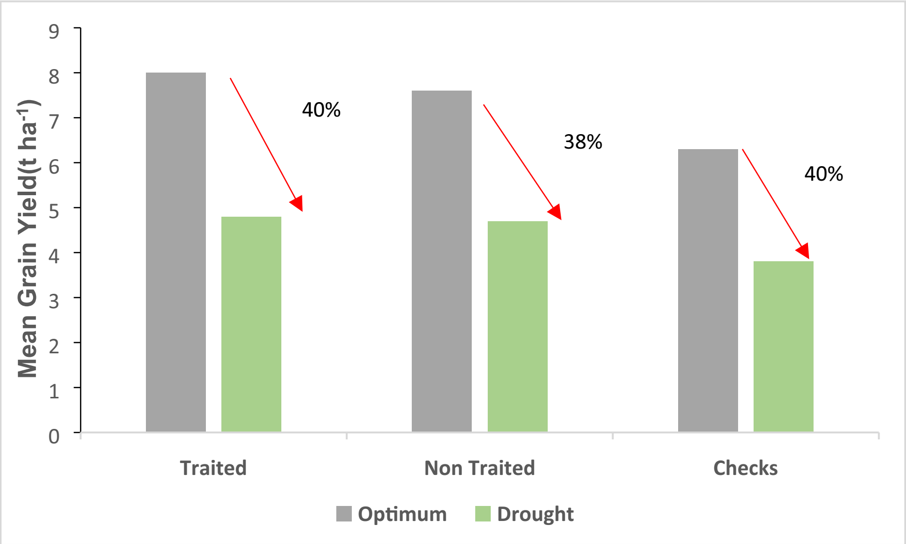

Fig. 5. Yield reduction of traited, non traited and check hybrids due to drought in Kenya. Percentages are yield reduction under drought relative to optimum-moisture condition; an indication of level of drought stress obtained in the trials.

pared with 0.80 to −0.35 t ha^{-1} (28.2 to −8.34%) under high drought stress (Fig. 7). One hybrid (WMA2003/WMB4401) showed a significantly greater yield increase of 28.2% due to the DT trait with no yield penalty under optimum-moisture condition (Fig. 7).

4.5.3. MON 87460 efficacy in South Africa

4.5.3.1. MON 87460 efficacy at Orania and Hopetown under optimum-moisture and drought stress. Under drought stress, differences between traited and non-traited hybrids ranged from 1.95 to −0.78 t ha^{-1} (37.3 to −14.0%) (Table 6, Fig. 8). Five traited hybrids had significant yield increase of 17.6–37.3% relative to the isohybrids under drought, and with no significant yield penalty under optimum-moisture condition (Fig. 8). Under optimum-moisture, one traited hybrid, CML312/CML395 had higher ($P < 0.05$) yield of 18% relative to the isogenic version, while two traited hybrids (CML312/S0125Z and S0158Z/S7852Z) had a significant yield penalty (Fig. 8).

Table 4
Pairwise comparison of yield for MON 87460 traited and non-traited hybrids under optimum-moisture condition in Kenya.

Pedigree	Traited N[1]	Non-traited N[1]	Traited Mean (t/ha)	Non-traited Mean (t/ha)	Delta (t/ha)	$LSD_{(0.05)}$ (t/ha)	Delta (%)	P-value
CML312/WMB4401	3	3	9.53	7.37	2.15	1.16	29.19	***
CML445/WMB4401	3	3	8.68	6.71	1.97	1.16	29.38	***
CML395/WMB4401	3	3	7.88	6.66	1.21	1.16	18.21	**
CML395/CML445	3	3	8.63	7.45	1.18	1.16	15.84	*
CML511/WMB4401	3	3	7.71	6.66	1.05	1.16	15.69	NS
CML442/WMB4401	3	3	7.78	7.18	0.60	1.16	8.31	NS
WMC8801/S0125Z	3	3	9.57	9.15	0.42	1.16	4.58	NS
CML312/CML445	3	2	8.72	8.53	0.20	1.16	2.31	NS
CML511/CML445	3	3	7.57	7.58	−0.01	1.16	−0.07	NS
WMC8801/WMB4401	3	3	9.04	9.09	−0.06	1.16	−0.61	NS
CML312/CML395	3	3	7.29	7.55	−0.26	1.16	−3.47	NS
WMA2003/WMB4401	3	3	7.74	8.16	−0.42	1.16	−5.14	NS
CML442/CML445	3	3	6.87	7.50	−0.62	1.16	−8.30	NS
CML442/CML395	3	3	5.37	6.20	−0.83	1.16	–	NS

[1] Number of data points used for statistical analyses; *** = significant at $P < 0.001$; ** = significant at $P < 0.01$; * = significant at $P < 0.05$; NS = Not significant at $P < 0.05$.

Table 5
Pairwise comparison of yield for MON 87460 traited and non-traited hybrids under managed drought in Kenya.

Pedigree	Traited N[1]	Non-traited N[1]	Traited Mean (t/ha)	Non-traited Mean (t/ha)	Delta (t/ha)	$LSD_{(0.05)}$ (t/ha)	Delta (%)	Significance
CML445/WMB4401	21	20	5.41	4.47	0.94	0.52	21.11	***
WMA2003/WMB4401	21	21	4.26	3.64	0.62	0.52	17.05	*
CML395/WMB4401	21	21	4.38	4.00	0.38	0.52	9.40	NS
CML511/CML445	21	21	4.84	4.56	0.28	0.52	6.10	NS
WMC8801/WMB4401	21	21	6.11	5.87	0.24	0.52	4.03	NS
CML511/WMB4401	21	21	4.82	4.59	0.23	0.52	5.02	NS
CML312/WMB4401	21	20	4.82	4.62	0.19	0.52	4.21	NS
CML442/CML395	21	19	3.15	2.97	0.18	0.52	6.12	NS
CML442/CML445	21	18	4.33	4.24	0.09	0.52	2.22	NS
CML312/CML395	21	21	4.61	4.67	−0.06	0.52	−1.19	NS
CML442/WMB4401	21	20	4.75	4.80	−0.06	0.52	−1.22	NS
CML395/CML445	21	21	4.94	5.05	−0.11	0.52	−2.15	NS
WMC8801/S0125Z	20	21	6.03	6.48	−0.44	0.52	−6.83	NS
CML312/CML445	20	20	5.27	5.97	−0.70	0.52	−11.74	**

[1] Number of data points used for statistical analyses; *** = significant at $P < 0.001$; * = significant at $P < 0.05$; NS = Not significant at $P < 0.05$.

4.5.3.2. MON 87460 efficacy at Lutzville under optimum-moisture and drought stress. The yield differences between traited and non-traited hybrids ranged from 2.95 to −1.38 t ha^{-1} (37.61 to −17.5%) under optimum-moisture condition and from 1.65 to −0.71 t ha^{-1} (35.9 to −15.4%) under drought stress (Fig. 9). Three traited hybrids (CML312/CML445; WMA8101/CML445; and CML312/S0125Z) had significantly more yield (significant %Delta) under drought stress. The MON 87460 gene had positive and significant effect (36–62%) under drought for these three hybrids without a significant yield penalty under optimum-moisture condition (Fig. 9).

5. Discussion

Overall efficacy of Event MON 87460 (*DroughtGard*®) evaluated in South Africa, Kenya, and Uganda showed that the gene had a positive effect on yield in most of the hybrids evaluated under managed stress conditions. Overall mean yield showed that the traited hybrids with MON 87460 drought gene yielded significantly higher than the non-traited, isohybrids. There was yield increase of 14–19% between traited and non-traited versions in 10% of the total hybrids evaluated across years, countries, and locations. Efficacy under the various drought stress levels showed that the MON 87460 traited hybrids had significantly higher yield (4–8%) under high-to severe-stress compared to low- to moderate -stress levels (0.4–0.7%).

These observations showed that MON 87460 gene conferred drought tolerance that reduced yield loss in traited maize hybrids over the non-traited, isohybrids, consistent with previous reports that MON 870460 transgenic trait's cold shock protein B (*CspB*) promotes drought tolerance by preserving normal plant functions under water stress[27,40]. Drought occurring during early seed development may cause the abortion of developing kernels, resulting in lower seed set or the shrinking of kernels, leading to yield and quality losses[69]. Field trials show that MON 87460 drought-tolerant transgenic hybrids have yield increase due to better grain set under drought conditions[54].

The number of data-points collected in this study in the high-stress environments was about 450 comparisons compared with severe-stress with only about 70 comparisons. The higher number of comparisons in the high stress environments contributed to the detection of a significant positive effect of the gene. The extensive testing in South Africa contributed to the greater expression of the gene compared with Kenya and Uganda, where there was limited number of trials due to limited number of permits received from the regulatory agencies for the confined field trials, few testing sites, and disruptions of managed drought stress by rains during the stressing periods.

The traited hybrids yielded better under high stress without yield penalty under optimum- moisture, suggesting that the drought gene conferred tolerance that protected grain yield loss. The fact that there was no significant yield difference observed between traited and non-traited hybrids under severe, moderate, and low drought stress but only at high stress level for same background germplasm tested, indicated that the responses to drought by the drought gene could be triggered only at certain stress levels in maize. Vinocur and Altman[62],

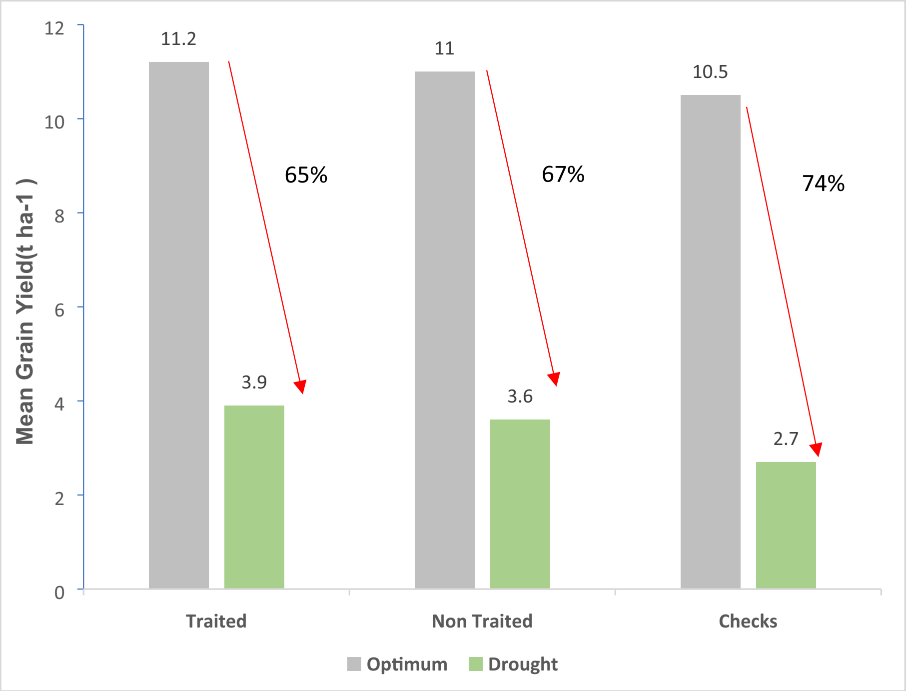

Fig. 6. Yield reduction of traited, non traited and check hybrids due to drought stress in Uganda; percentages are yield reduction due to drought stress relative to optimum-moisture condition; an indication of level of drought stress obtained in the trials.

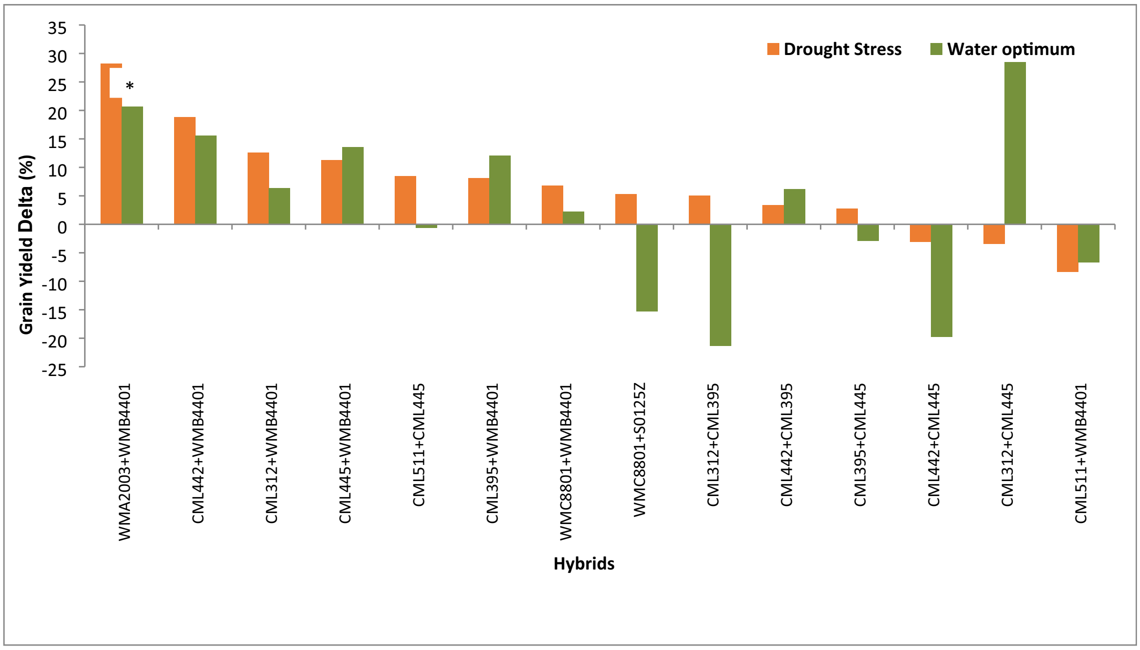

Fig. 7. Percentage yield differences between traited and no-traited hybrids under drought and optimum-moisture conditions in Uganda; * = significant at $P < 0.05$.

reported that transgenes introduced to a target crop modifies the signaling of transduction pathways to activate stress responses that regulate adaptation mechanisms to drought. MON 87460 traited hybrids are known to yield better under drought stress without yield penalty under optimum-moisture[37,61]. The ability to protect yield loss in higher stress environments, without yield penalty in low stress and high yield potential environments as reported by Adee et al.[1] will benefit farmers in rainfed areas in Africa, where sporadic drought stress levels vary considerably over the growing seasons, with greater impacts during flowering and grain filling.

Different maize genotypes adapt to drought stress in different ways due to genetic background diversity[18,7], hence the variability in this study. Most of the hybrids used in the study showed significant yield improvement due to the gene, and thus, there is a great potential in the improvement of drought tolerance by incorporating the gene in diverse germplasm in a maize breeding program. Drought tolerant

Table 6
Pairwise comparison of yield for MON 87460 traited and non-traited hybrids under drought stress at Orania and Hopetown in South Africa.

Pedigree	Traited N[1]	Non-traited N[1]	Traited Mean (t/ha)	Non-traited Mean (t/ha)	Delta (t/ha)	LSD$_{(0.05)}$ (t/ha)	Delta (%)	Significance
WMA2003/WMB4401	23	23	7.17	5.22	1.95	1.98	37.33	***
CML489/WMB4401	23	23	6.07	4.67	1.40	1.98	29.95	*
CML442/CML445	23	23	5.68	4.51	1.17	1.98	25.92	*
CML511/CML445	23	23	5.21	4.22	0.99	1.98	23.35	NS
CML442/CML312//CML445	23	23	5.91	4.81	1.10	1.98	22.81	*
CML488/WMB4401	23	22	6.08	5.03	1.05	1.98	20.96	NS
CML442/CML312//WMB4401	23	23	6.17	5.13	1.04	1.98	20.33	NS
WMA2001/CML445	23	23	7.48	6.36	1.12	1.98	17.62	*
WMA2003/CML395	23	22	5.81	4.98	0.84	1.98	16.77	NS
CML395/CML445	23	23	6.85	6.05	0.80	1.98	13.17	NS
CML204/WMB4401	23	23	5.39	4.82	0.58	1.98	11.96	NS
WMA8101/CML445	23	23	6.98	6.38	0.59	1.98	9.29	NS
WMA2001/WMB4401	23	23	7.18	6.70	0.48	1.98	7.18	NS
CML442/CML312//CML395	23	23	5.23	5.03	0.20	1.98	3.97	NS
CML511/WMB4401	23	23	5.92	5.76	0.16	1.98	2.81	NS
WMC8801/S0125Z	23	23	9.09	8.88	0.21	1.98	2.37	NS
CML312/S0125Z	23	22	7.92	7.85	0.06	1.98	0.80	NS
CML312/CML445	22	22	6.59	6.54	0.05	1.98	0.78	NS
S9733Z/S7852Z	23	23	7.83	7.82	0.01	1.98	0.14	NS
S7607Z/S7852Z	23	23	7.87	7.87	0.00	1.98	−0.03	NS
WMA2001/CML395	23	23	6.20	6.22	−0.02	1.98	−0.29	NS
S0158Z/S7852Z	23	23	6.88	7.06	−0.18	1.98	−2.57	NS
CML312/CML395	23	22	4.82	5.60	−0.78	1.98	−13.97	NS

[1] Number of data points used for statistical analyses *** = significant at $P < 0.001$; * = significant at $P < 0.05$; NS = Not significant at $P < 0.05$.

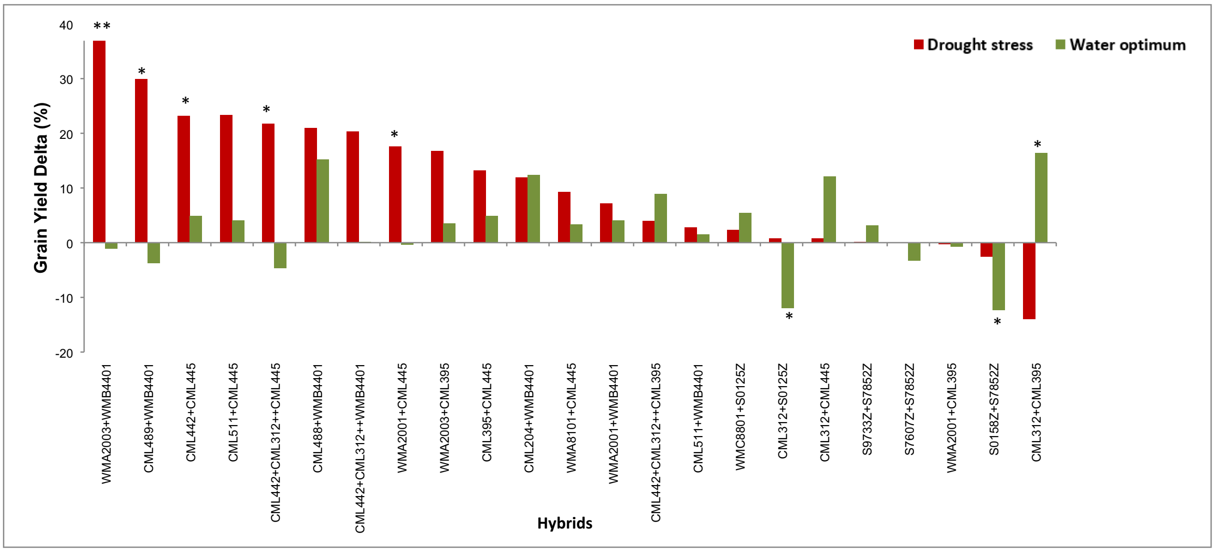

Fig. 8. Percentage yield differences between traited and no-traited hybrids under drought and optimum-moisture conditions at Orania and Hopetown in South Africa; * = significant at $P < 0.05$; ** = Significant at $P = 0.01$.

maize genotypes exhibit adaptation for resilience to moisture stress to favour plant phenology, normal growth and development, good grain filling, and translocation of assimilates[18] hence less yield loss, compared to susceptible genotypes that lack adaptive mechanisms. Studies on MON 87460 maize showed that the plants exhibited significant relative increases in ear length, kernel number, and grain weight as adaptation mechanism to water stress[21]. Drought tolerance is a complex mechanism controlled by interaction of various biochemical factors and pathways during plant growth process[6].

Results of multilocation yield trials comparing *DroughtGard®* (MON 87460; *CspB*) hybrid maize and non-transgenic control showed 6% yield increase of transgenic maize over non-transgenic versions over years[40]. In the current study, on average, yield increase of 14 to 19% between traited hybrids and non-traited versions was achieved in 10% of all the hybrids evaluated. Further, across South Africa, Kenya, and Uganda, for three or more years, five traited hybrids had 7 to13% significantly higher yield than the non-traited isohybrids versions out of 34 adapted DroughtTEGO® hybrids that were previously developed as drought tolerant through conventional breeding accelerated using marker assisted recurrent selection[11]. These results demonstrated the efficacy of the DT Event MON 87460 with yield increase greater than the expected 8–10% yield increase envisaged in the original product concept[44]; thus, demonstrating that the DT gene works in tropical African germplasm.

The yield increase under water-limited conditions was reported to be consistent and significantly different between MON 87460 traited and non-traited hybrids and in support of the product concept[52]. Moisture stress at the critical reproductive stage of two weeks before, to two weeks after flowering has a significant grain yield reduction in maize[63,42,41,65]. Agronomic traits evaluated by USDA–APHIS[61] showed

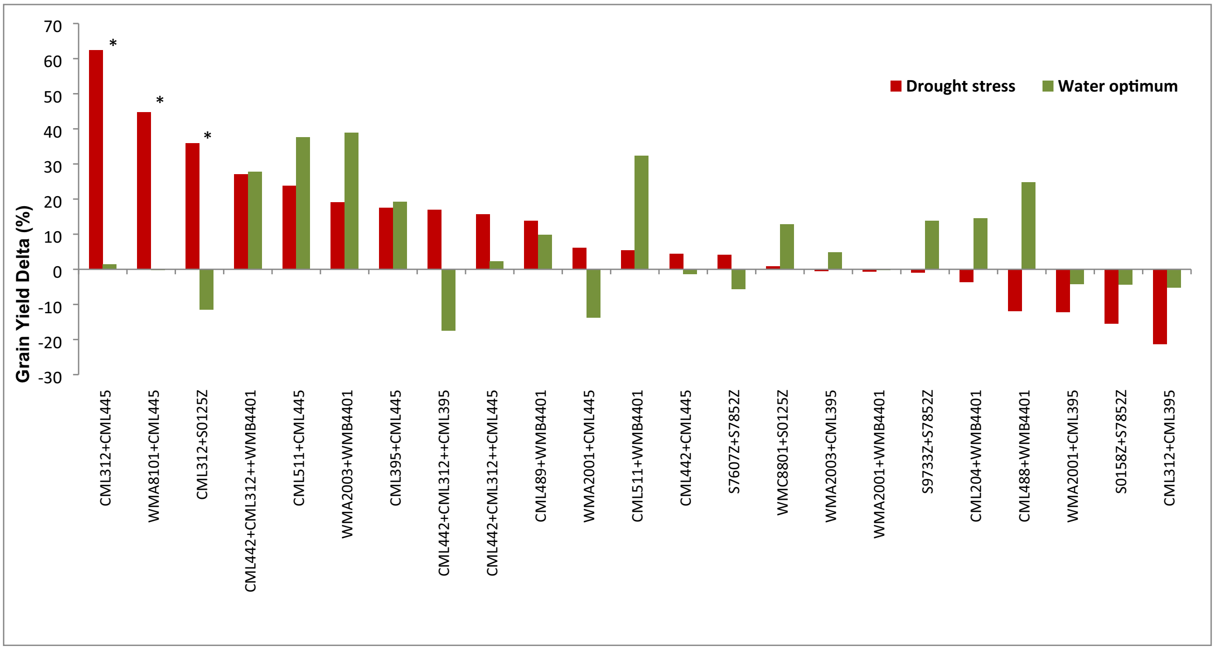

Fig. 9. Percentage yield differences between traited and non-traited hybrids under drought stress and optimum-moisture at Lutzville, South Africa; * = significant at $P < 0.05$.

that MON 87460 maize hybrids are the same as conventional maize in terms of pest potential, the ability to establish as a weed, ecological interactions with non-target organisms, and tolerance or susceptibility to abiotic stressors other than tolerance to drought stress. This suggests that MON 87460 transgenic maize has benefits of protecting farmers' yield in stress-environments, and ecologically safe for cultivation.

The fact that most hybrids had a significant positive effect on yield while some did not, indicated that interaction of gene with germplasm in various environments may be important in harnessing and selecting transgenic drought tolerant genotypes when the trait is deregulated for open cultivation. In some instances, the actual stress imposed was higher than the expected target stress levels, resulting in very low yield for both traited and non-traited hybrids. This showed that severe or chronic drought stress equally affects both MON 87460 transgenic and conventional maize yield that can be reduced to zero as previously reported by Lybbert and Bell[31] and Lybbert and Carter[32].

Developing drought stress tolerant improved crop varieties through transgenics has many practical limitations that could reduce the effectiveness of the transgenes due to side effects and complexity of the tolerance mechanisms[25,13]. Maize has a set of multiple genes that control complex physiological responses which vary with timing, duration, and severity of drought[18,34,7]. Altering one or few genes may not only confer drought tolerance but could also lead to yield penalty. Therefore, selecting transgenic drought tolerant varieties under drought stress and without yield penalty under optimum moisture condition, is key to identifying suitable varieties for farmers to manage the impact of frequent drought events caused by climate change in Africa.

6. Conclusion

MON 87460 had a positive gene effect on most of the maize hybrids tested in Kenya, Uganda and South Africa across countries, years, and locations. The gene had a positive yield effect (efficacious) on 50% of the 120 adapted and non-adapted maize hybrids. The efficacy of DT MON 87460 was highest in Lutzville, South Africa with positive and significant gene effect on grain yield increase of 36 to 62% in three traited single-cross hybrids relative to their non-traited versions under drought, and without yield penalty under optimum-moisture conditions. In the 34 adapted hybrids with same base genetics evaluated for ≥ 3 years, across the three countries, five traited hybrids had 7 to 13% greater yield than their non-traited versions. The MON 87460 gene had a significant positive effect on yield of hybrids under high stress compared with severe, moderate, and low stress levels at all the locations.

This study showed that when MON 87460 transgene is deregulated for open-cultivation in Africa and used to develop drought-tolerant hybrids and widely evaluated across maize growing agroecologies for yield performance and stability, it can effectively improve drought tolerance in maize and cushion farmers against yield loss due to frequent drought events caused by climate change. However, the ability to successfully test and select for MON 87460 traited genotypes for drought tolerance is difficult, thus germplasm with the gene should be tested widely across agroecologies and years for their yield performance and stability to identify the desirable drought tolerant varieties for release to farmers for cultivation.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The data that support the findings of this study are available from Bayer Crop Science, but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of Bayer Crop Science.

Funding

This work was supported by the Bill and Melinda Gates Foundation, the Howard G. Buffett Foundation, and the United States Agency for International Development through the Water Efficient Maize for Africa (WEMA) Project (Contract ID: OPP1019943).

CRediT authorship contribution statement

Caleb O. Obunyali: Writing – original draft, Writing- review & editing. **Kiru Pillay:** Data curation, Formal analysis, Investigation, Methodology. **Barbara Meisel:** Data curation, Formal analysis, Investigation, Methodology. **Eric N Ndou:** Data curation, Investigation, Methodology, Writing – review & editing. **Kingstone Mashingaidze:** Investigation, Supervision, Writing – review & editing. **Julius Pyton Sserumaga:** Data curation, Investigation, Methodology, Writing – review & editing. **Godrefy Asea:** Investigation, Supervision, Writing – review & editing. **Murenga Mwimali:** Data curation, Investigation, Supervision, Writing – review & editing. **Regina Tende:** Data curation: Methodology, Supervision, Writing – review & editing. **Yoseph Beyene:** Investigation, Supervision, Writing – review & editing. **Stephren Mugo:** Investigation, Methodology, Supervision, Writing – review & editing. **Emmanuel Okogbenin:** Writing – review & editing. **Sylvester O Oikeh:** Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to thank the WEMA Project partners that jointly implemented the study: Kenya Agricultural Research Organization (KALRO) in Kenya; National Research Organization (NARO) in Uganda; Agricultural Research Council (ARC) in South Africa; the International Maize and Wheat Improvement Center (CIMMYT), Bayer Crop Science, and AATF. The various government regulators and agencies that facilitated and guided the implementation of Confined Field Trials (CFTs) activities at the various sites in compliance with CFT permit regulations and approvals for the studies in the project countries are also appreciated. Lastly, we would like to thank the Bill and Melinda Gates Foundation, the Howard G. Buffett Foundation and the United States Agency for International Development (USAID) who supported this research work through the Water Efficient Maize for Africa (WEMA) Project (Contract ID: OPP1019943).

Appropriate permissions and/or licences for the study: The studies were conducted in line with the Biosfatey laws in each country where the required CFT permits were obtained from the concerned regulators.

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