



## Research article

# Isogenic genetically-modified and non-genetically-modified corn yields and response to drought and N rates

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## Abstract

Trait integration into conventional hybrids is done *via* marker-assisted backcrossing on one or both parent lines to achieve near isogenic lines. The parent lines for genetically modified (GM) hybrids were selected based on equivalence in genetics, phenotype and field performance to their conventional counterpart. The response was investigated of two GM variants of a hybrid (herbicide-tolerant Dekalb® 6818R, with transformation event, NK603; herbicide-tolerant and insect-resistant Dekalb® 6818S, with MON89034 × NK603 events “stack”) and its near-isogenic non-GM variant (Dekalb® 6818C) under different water and N conditions. Water, N and their interaction showed significant effects on yield and agrophysiological traits. Combining full irrigation ( $W_3$ ) with 180 kg N/ha maximized the mean ( $\pm$  SD) yield ( $9,990 \pm 318$  kg/ha) and biomass ( $2.04 \pm 0.23$  kg/m<sup>2</sup>). Variant as a main effect did not significantly impact yield and agrophysiological traits but isogene interaction with water and N showed a  $G_2$  ( $2.14 \pm 0.22$  kg/m<sup>2</sup>) significant advantage over  $G_1$  ( $2.00 \pm 0.29$  kg/m<sup>2</sup>) and  $G_0$  ( $2.02 \pm 0.30$  kg/m<sup>2</sup>) in accumulating pre-anthesis biomass under  $W_3$  across N rates. Under moderate drought ( $W_2$ ) with 0 N,  $G_2$  had higher current assimilate transfer to kernels ( $642.51 \pm 83.21$  g/m<sup>2</sup>), percentage contribution of current assimilates to kernel ( $71.13 \pm 2.09$  %) and yield ( $4,950 \pm 768$  kg/ha) than  $G_1$  and  $G_0$ . Under prolonged drought ( $W_1$ ) with 180 kg N/ha, the  $G_2$  yield ( $7,484 \pm 145$  kg/ha) was 9% higher than for  $G_1$  ( $6,858 \pm 105$  kg/ha) and  $G_0$  ( $6,870 \pm 208$  kg/ha).

## Introduction

Marker-assisted backcrossing has been used in the introgression of transgenes into elite inbred lines, which become the parents of the commercially available GM corn

hybrids (Venkatesh et al., 2015). All GM progenies derived from crosses of conventionally bred corn are screened over several generations and diverse environments to ensure that the desired traits are phenotypically expressed, stable and heritable under a wide range of environmental conditions (Laurena, 2005). A primary requirement for commercializing

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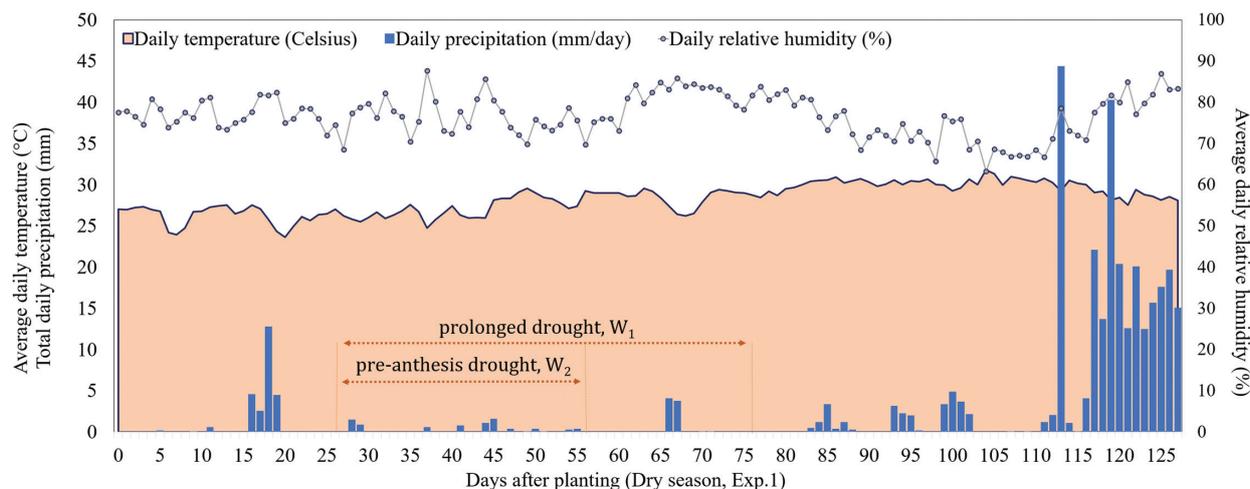
a GM crop is substantial equivalence that agrees to the guidelines of international governing bodies (Organization for Economic Cooperation and Development, United Nations Food and Agriculture Organization, World Health Organization and Codex Alimentarius Commission) and involves comparative assessment for agronomic, morphological, chemical and nutritive characteristics (The International Service for the Acquisition of Agri-biotech Applications, 2018). The Philippines was the first Asian country to commercialize GM corn in 2002, and adoption has since increased to a peak of 831,000 ha in 2014, with “stacks”—latest technology combining glyphosate-tolerant and lepidopteran pest damage resistance transgenes—occupying 92% of the total planted area (The International Service for the Acquisition of Agri-biotech Applications, 2018). Most new hybrids, which have been selected for high yield under high inputs, are faced with the evolutionary trade-off between high productivity and adaptability to water and N-limited conditions (Venkatesh et al., 2015). Previous studies have shown genetic variations on plant responses to drought and N stress—hybrids that are ‘staygreen’, that can produce higher pre-anthesis root and shoot biomass and that can remobilize assimilate reserves from vegetative parts to the developing embryo (kernel) can achieve higher grain yields under water and N deficiency (Moser et al., 1998; Pandey et al., 2000; Molla et al., 2014). There has been no current report on a field study comparing the responses of tropical near-isogenic GM and non-GM corn hybrids to different water and N levels, particularly on the isogenes’ capacity to accumulate pre-anthesis biomass, to allocate vegetative biomass to reproductive organs and to produce grain yield and harvest biomass. The current tested hypothesis was that water, N and their synergistic interaction wield far greater influence on yield and response than the isogene and its interaction to either or both water and N. In addition, it was assumed that adequate water improves soil N availability, root uptake and yield response to N fertilization when N is sub-optimal, while an increased N fertilizer rate can improve water use efficiency and mitigate the detrimental impacts of drought on plant development.

## Materials and Methods

Two field experiments were conducted in 2016 in Victoria, Tarlac, the Philippines (15° 33′ 6.56″ N, 120° 39′ 12.51″ E). This vertisol soil type (69.13% coarse sand, 8.49% medium–fine sand, 19.38% silt and 3.00% clay) has an organic matter content of 0.92%, a pH 5.61 and an aqueous soil moisture due

to volcanic sediments deposition of the Moncada Series. Three near-isogenic hybrids (G) were studied: Dekalb® 6818 germplasm from Monsanto Company, non-transgenic Dekalb® 6818C ( $G_0$ ), glyphosate-tolerant Dekalb® 6818R with transformation event, NK603 ( $G_1$ ) and glyphosate-tolerant and lepidopteran pest damage-resistant Dekalb® 6818S—stacked with two transformation events, MON89034 × NK603 ( $G_2$ ). The first experiment (Exp.1) was conducted in the dry season (January to May) to compare G under three W regimes [prolonged drought, bracketing flowering ( $W_1$ ); pre-anthesis drought ( $W_2$ ); and well-irrigated ( $W_3$ )] and three N rates [0 kg/ha ( $N_1$ ), 90 kg/ha ( $N_2$ ), 180 kg/ha ( $N_3$ )]. Ensuring that primary plant nutrients phosphorous (P) and potassium (K) were kept constant across treatments, while only varying N, the following NPK fertilizer levels were used for  $N_0$ ,  $N_1$  and  $N_2$ , respectively: [0-21-21 (basal application at sowing)], [21-21-21 (basal application at sowing) + 69 kg N/ha (side dressed at 25 days after planting, DAP)] and [90-21-21 (basal at sowing) + 69 kg N/ha (25 DAP) + 21 kg N/ha (35 DAP)]. The treatments were laid out in a split-split plot design (W as main plot, N as subplot and G as sub-subplots), with three replications, and plots of 10 rows by 5 m length. A second experiment (Exp.2) was conducted under rain-fed conditions (July to November) using a split-plot design (N as main plot and G as subplot) with four replications. The seeding rate was 83,000 plants/ha for both experiments. Pre-emergent herbicide (pendimethalin at 330 g ae/L) was sprayed at the recommended rate of 3 L/ha at 2 DAP to ensure a weed-free seed bed. Weeds were controlled using glyphosate (Roundup® TurboPlus™ 540 g (acid equivalent, ae/L) for  $G_1$  and  $G_2$  plots, following the label recommendations, while manual weeding was used for  $G_0$  and any GM plots adjacent to  $G_0$  to avoid unintended herbicide phytotoxicity on non-GM plants.

$W_1$ ,  $W_2$  and  $W_3$  plots were synchronously irrigated every 10 d from sowing until 30 DAP, after which drought treatments were imposed: withholding irrigation for 30 days (30–60 DAP) until tasseling (VT) for  $W_2$ ; and for 50 days (30–80 DAP) until milk stage (R3) for  $W_1$ .  $W_3$  plots were irrigated until 90 DAP at a 10 d average interval, which was shortened when soil tensiometer readings approached 40 centibars. The experiment was conducted under farmer field conditions and no rainout shelter was employed. Nevertheless, the average daily (0.27 mm) and total ( $W_2 = 5.80$  mm;  $W_1 = 13.90$  mm) precipitation amounts during drought imposition were negligible, while the average temperature and relative humidity for the same period were 27.9°C and 79%, respectively (Fig. 1). At physiological maturity, a 12 m<sup>2</sup> plot was hand-



**Fig. 1** Average daily temperature, daily relative humidity and total daily precipitation January–May 2016 (Exp.1)

harvested to measure the parameters, kernel moisture content (MST), shelling percentage (SP), total ear count (TEC) and final stand count (FSC), from which grain yield (YLD, in kilograms per hectare) was calculated. Twenty ears per plot were used to measure kernel number (KN) per square meter, kernel yield per square meter (KY), 100-kernel weight ( $KW_{100}$ ), ear length (EL) and ear diameter (ED). For dry matter determination, three plant samples, including roots, were randomly selected from each plot (Molla et al., 2014). The plant parts were separated into roots, stalk, leaves, ear, husk and tassel and then oven-dried at 70°C until constant weight. Each plant part was weighed and the total plant dry weight per square meter at anthesis ( $TPDW_a$ ) and at harvest ( $TPDW_h$ ) were calculated. Assuming that the dry matter lost from  $TPDW_a$  was translocated to the developing kernel, assimilate partitioning is the most critical translocation process that determines plant productivity (Singletary and Below, 1990). Hence the agrophysiological parameters were evaluated following Molla et al. (2014): current assimilate transfer to kernel [(CATK) =  $KY - (TPDW_a - TPDW_h)$ ], which measures the amount of assimilates transferred from green leaves to the developing kernels; contribution of current assimilates to kernel [(CCAK) =  $CATK / KY \times 100\%$ ]; contribution of pre-anthesis dry matter to kernel [(CPDMK) =  $(TPDW_a - TPDW_h) / KY \times 100\%$ ]. Analysis of variance used the JMP® 12.0.0 software (SAS Institute, 2015) to assess the main and interaction effects of the treatments, while Student's t test was used for pair-wise treatment comparisons. All statistical tests were considered significant when  $p < 0.05$ .

## Results and Discussion

### Yield and yield components

#### *Effects of water (W), nitrogen (N) and isogenes (G) on yield and yield components*

Water showed a strong positive impact on yield (YLD) in Exp.1, with the well-irrigated ( $W_3$ ) plots producing 8% and 46% higher YLDs than pre-anthesis ( $W_2$ ) and prolonged drought ( $W_1$ ) plots, respectively (Table 1). Reduction in number of kernels (KN; Table 1) when subjected to pre-anthesis (7%) and prolonged (45%) droughts compared to full irrigation demonstrated KN's suitability as an indicator of drought impact on YLD (Kebede et al., 2014). Water also had a significant effect on the shelling percentage (SP; Table 1):  $W_3$ -SP was 1% and 5% higher than  $W_2$ - and  $W_1$ -SPs, respectively. No significant differences were detected between well-irrigated and pre-anthesis drought plots on ear diameter (ED), 100 kernel weight ( $KW_{100}$ ), kernel yield (KY), final stand count (FSC) and total ear count TEC; but prolonged drought plots showed significantly lower values on all five components (−3% ED, −15%  $KW_{100}$ , −73% KY, −4% FSC, −9% TEC) versus both  $W_3$  and  $W_2$  (Table 1). N had a significant effect on YLD and all components in Exp.1, and in most components [excluding SP, ED and ear length (EL)] in Exp. 2 (Table 1). A significant  $N_3 > N_2 > N_1$  trend was observed in YLD,  $KW_{100}$  and TEC for Exp.1, and in KN and KY for Exp.2. In Exp.1, YLD was maximized (8.7 MT/ha) at  $N_3$ -10% and -105% higher than  $N_2$ - and  $N_1$ -LDs, respectively; in Exp.2, maximum YLD (8.8 MT/ha) was achieved using 90 kg N/ha ( $N_2$ ), with no incremental YLD achieved with doubling the rate ( $N_3$ ),

but both the yields from N<sub>2</sub> and N<sub>3</sub> were 88% higher than the N<sub>1</sub>-YLD. The Our two experiments revealed that YLD reduction in no-N plots was largely due to increased kernel abortion and fewer kernels per ear, caused by the decreased capacity of the developing kernel to allocate and metabolize sugars for protein and starch biosynthesis (Singletary and Below, 1990). The yield parity between N<sub>2</sub> and N<sub>3</sub> (Exp.2) has been explained in other studies (Pandey et al., 2000; Molla et al., 2014) as a manifestation of corn's highly efficient N-recovery under favorable conditions and lower efficiency in poor growing conditions. This N<sub>3</sub> = N<sub>2</sub> > N<sub>1</sub> trend was also observed in other yield components in both experiments. In Exp.1, applying 90–180 kg N/ha resulted in the following significant increases in yield components versus no-N: 22% KN, 67% KY, 8% FSC, 10% EL, 5% ED and 3% SP. In Exp.2, both N<sub>3</sub> and N<sub>2</sub> had equally higher KW<sub>100</sub> (26%), FSC (5%)

and TEC (8%) than N<sub>1</sub>. Isogene, as a main effect, significantly impacted YLD in Exp.2 but not in Exp.1 (Table 1). The absence of G<sub>2</sub> transgenes' target insect pests (Asian corn borer (*Ostrinia furnacalis*), corn earworm (*Helicoverpa spp*) and common cutworm (*Spodoptera spp*); The International Service for the Acquisition of Agri-biotech Applications, 2018) in all plots would explain the YLD parity among the isogenes in Exp.1. In Exp.2, the 6% YLD advantage of both transgenic hybrids over G<sub>0</sub> could be attributed to the benefit of using glyphosate on glyphosate-tolerant corn in managing weeds in the rainy season, when weeds can grow faster and are more competitive for nutrients. Isogene also significantly influenced ear sizes in Exp.1 (ED values for G<sub>1</sub> and G<sub>2</sub> were 2% bigger than for G<sub>0</sub>, while EL for G<sub>2</sub> was 5% and 13% longer than for G<sub>1</sub> and G<sub>0</sub>, respectively) and kernel number in Exp.2 (N values for G<sub>2</sub> and G<sub>0</sub> were 3% higher than for G<sub>1</sub>).

**Table 1** Main and interaction effects of water (W), nitrogen (N) and isogenes (G) on yield (YLD), shelling percentage (SP), ear diameter (ED), ear length (EL), kernel number (KN), kernel weight (KW<sub>100</sub>), kernel yield (KY), final stand count (FSC) and total ear count (TEC) in 2016 dry (Exp.1) and wet (Exp.2) seasons

Treatment	YLD (kg/ha)	SP (%)	ED (cm)	EL (cm)	KN (/m <sup>2</sup> )	KW <sub>100</sub> (g)	KY (g/m <sup>2</sup> )	FSC (count)	TEC (count)
<i>Main Effects</i>									
Exp.1									
Water (W)	**	**	**	ns	**	**	**	**	**
W <sub>1</sub>	5,350 <sup>c</sup>	76.80 <sup>c</sup>	7.23 <sup>b</sup>	16.56	3,193 <sup>c</sup>	21.40 <sup>b</sup>	658 <sup>b</sup>	7.71 <sup>b</sup>	7.47 <sup>b</sup>
W <sub>2</sub>	7,245 <sup>b</sup>	81.00 <sup>b</sup>	7.48 <sup>a</sup>	17.24	4,632 <sup>b</sup>	24.87 <sup>a</sup>	1,177 <sup>a</sup>	8.03 <sup>a</sup>	8.07 <sup>a</sup>
W <sub>3</sub>	7,790 <sup>a</sup>	81.58 <sup>a</sup>	7.39 <sup>a</sup>	16.83	4,974 <sup>a</sup>	25.34 <sup>a</sup>	1,264 <sup>a</sup>	8.00 <sup>a</sup>	8.13 <sup>a</sup>
Nitrogen (N)	**	**	**	**	**	**	**	**	**
N <sub>1</sub>	3,924 <sup>c</sup>	78.31 <sup>b</sup>	7.12 <sup>b</sup>	15.81 <sup>b</sup>	3,722 <sup>b</sup>	18.61 <sup>c</sup>	715 <sup>b</sup>	7.53 <sup>b</sup>	7.18 <sup>c</sup>
N <sub>2</sub>	7,771 <sup>b</sup>	80.47 <sup>a</sup>	7.45 <sup>a</sup>	17.06 <sup>a</sup>	4,574 <sup>a</sup>	25.36 <sup>b</sup>	1,153 <sup>a</sup>	8.10 <sup>a</sup>	8.11 <sup>b</sup>
N <sub>3</sub>	8,690 <sup>a</sup>	80.62 <sup>a</sup>	7.53 <sup>a</sup>	17.76 <sup>a</sup>	4,504 <sup>a</sup>	27.64 <sup>a</sup>	1,229 <sup>a</sup>	8.11 <sup>a</sup>	8.38 <sup>a</sup>
Isogenes (G)	ns	ns	**	**	ns	ns	ns	ns	ns
G <sub>0</sub>	6,725	79.68	7.28 <sup>b</sup>	15.75 <sup>c</sup>	4,295	24.94	1,066	7.91	7.91
G <sub>1</sub>	6,750	79.69	7.48 <sup>a</sup>	17.02 <sup>b</sup>	4,281	23.36	1,025	7.93	7.88
G <sub>2</sub>	6,911	80.03	7.34 <sup>b</sup>	17.86 <sup>a</sup>	4,224	23.31	1,007	7.91	7.88
Exp.2									
Nitrogen (N)	**	ns	ns	ns	**	**	**	**	**
N <sub>1</sub>	4,665 <sup>b</sup>	81.31	7.36	16.64	4,237 <sup>c</sup>	21.13 <sup>b</sup>	926 <sup>c</sup>	7.77 <sup>b</sup>	7.72 <sup>b</sup>
N <sub>2</sub>	8,943 <sup>a</sup>	82.00	7.57	17.69	5,242 <sup>b</sup>	27.59 <sup>a</sup>	1,705 <sup>b</sup>	8.15 <sup>a</sup>	8.34 <sup>a</sup>
N <sub>3</sub>	8,789 <sup>a</sup>	81.82	7.39	16.94	5,667 <sup>a</sup>	25.49 <sup>a</sup>	1,876 <sup>a</sup>	8.11 <sup>a</sup>	8.29 <sup>a</sup>
Isogenes (G)	*	ns	ns	ns	*	ns	ns	ns	ns
G <sub>0</sub>	7,816 <sup>b</sup>	81.65	7.42	16.62	5,161 <sup>a</sup>	24.63	1,515	7.98	8.09
G <sub>1</sub>	8,330 <sup>a</sup>	81.50	7.39	16.89	4,952 <sup>b</sup>	24.93	1,490	8.01	8.06
G <sub>2</sub>	8,251 <sup>a</sup>	81.98	7.52	17.75	5,033 <sup>ab</sup>	24.65	1,500	8.04	8.20
<i>Interaction Effects</i>									
W × N × G	*	ns	**	**	ns	**	**	**	ns
W × N	**	**	**	**	**	*	**	**	**
W × G	ns	ns	ns	ns	ns	ns	ns	ns	ns
N × G (Exp.1)	ns	ns	**	**	ns	*	**	ns	ns
N × G (Exp.2)	ns	ns	ns	ns	**	**	**	ns	*

W<sub>1</sub> = prolonged drought, W<sub>2</sub> = pre-anthesis drought, W<sub>3</sub> = well-irrigated, N<sub>1</sub> = 0 kg N/ha, N<sub>2</sub> = 90 kg N/ha, N<sub>3</sub> = 180 kg N/ha, G<sub>0</sub> = non-transgenic Dekalb® 6818C, G<sub>1</sub> glyphosate-tolerant Dekalb® 6818R, G<sub>2</sub> = glyphosate-tolerant and lepidopteran pest-resistant Dekalb® 6818S;

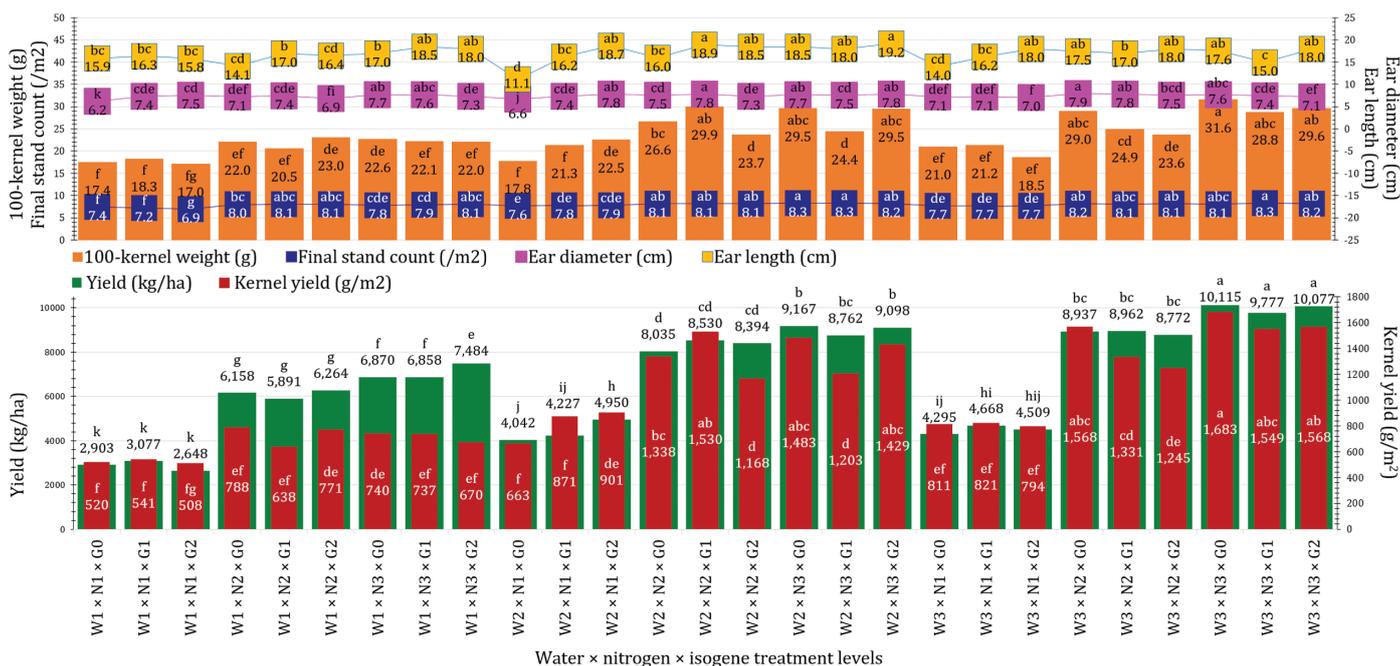
\*, \*\* denote significance ( $p < 0.05$ ) and high significance ( $p < 0.01$ ), respectively; ns = non-significance ( $p > 0.05$ ); Means in a column within each main effect superscripted with different lowercase letters are significantly ( $p < 0.05$ ) different.

*Interaction effects of water (W), nitrogen (N) and isogenes (G) on yield and yield components*

The interaction of W, N and G significantly impacted YLD, KW<sub>100</sub>, KY, FSC, ED and EL (Table 1). When interacting with W and N, isogene played a lesser role in impacting YLD when both were at optimum or at extreme deficit levels, and a more prominent role when one was severely deficient and the other adequate (Fig. 2). YLD was highest (approximately 10 t/ha) in W<sub>3</sub> × N<sub>3</sub> × (G<sub>2</sub>, G<sub>1</sub>, G<sub>0</sub>), and lowest (approximately 2.9 t/ha) in W<sub>1</sub> × N<sub>1</sub> × (G<sub>2</sub>, G<sub>1</sub>, G<sub>0</sub>). Under the stress of W<sub>2</sub> and N<sub>1</sub>, G<sub>2</sub> yielded 17% more than both G<sub>1</sub> and G<sub>0</sub>, while its ear diameter (5% and 17%) and length (16% and 68%) were also greater than for G<sub>1</sub> and G<sub>0</sub>, respectively (Fig. 2). Under W<sub>1</sub> and N<sub>3</sub> conditions, the YLD from G<sub>2</sub> was 8% higher than for both G<sub>1</sub> and G<sub>0</sub>, due to 3% more harvested plants (FSC; Fig. 2). KW<sub>100</sub> and KY followed a similar trend as YLD (Fig. 2); plots with adequate W and N across isogenes recorded the highest values for KW<sub>100</sub> (approximately 30 g) and KY (approximately 1.6 kg/m<sup>2</sup>), while plots with either

W or N, or both, in severe deficit had the lowest values for KW<sub>100</sub> (19 g) and KY (591 g/m<sup>2</sup>), except for KY for W<sub>3</sub> × N<sub>1</sub> × (G<sub>2</sub>, G<sub>1</sub>, G<sub>0</sub>), which showed the ameliorative effect of adequate water under no N.

Isogene showed a more pronounced role in interacting with W and N in impacting FSC, ED and EL than other yield components (Fig. 2). The FSC for W<sub>1</sub> × N<sub>1</sub> × G<sub>2</sub> was the lowest across all treatments, and 6% lower than for both G<sub>0</sub> and G<sub>1</sub> under W<sub>1</sub> and N<sub>1</sub>. The biggest ear size (approximately 18.4 cm EL × 7.8 cm ED) was recorded from W<sub>3</sub> × N<sub>3</sub> × G<sub>0</sub>, W<sub>3</sub> × N<sub>2</sub> × G<sub>0</sub>, W<sub>2</sub> × N<sub>3</sub> × G<sub>0</sub>, W<sub>2</sub> × N<sub>2</sub> × G<sub>2</sub>, W<sub>2</sub> × N<sub>3</sub> × G<sub>2</sub>, W<sub>2</sub> × N<sub>1</sub> × G<sub>2</sub>, W<sub>2</sub> × N<sub>2</sub> × G, and W<sub>1</sub> × N<sub>3</sub> × G<sub>1</sub>. The EL value for W<sub>2</sub> × N<sub>1</sub> × G<sub>0</sub> was the shortest (11.1 cm); and ED for W<sub>1</sub> × N<sub>1</sub> × G<sub>0</sub> was the narrowest (6.2 cm). These values demonstrated that isogenes can produce optimum ear sizes under different W × N conditions: for G<sub>2</sub> under varying N rates and adequate W; for G<sub>1</sub> under varying W levels but with adequate N; and for G<sub>0</sub> under adequate W and N.



**Fig. 2** Interaction effects of water (W), nitrogen (N) and isogenes (G) on yield (YLD), kernel weight (KW<sub>100</sub>), kernel yield (KY), final stand count (FSC), ear diameter (ED) and ear length (EL), where W<sub>1</sub> = prolonged drought, W<sub>2</sub> = pre-anthesis drought, W<sub>3</sub> = well-irrigated, N<sub>1</sub> = 0 kg N/ha, N<sub>2</sub> = 90 kg N/ha, N<sub>3</sub> = 180 kg N/ha, G<sub>0</sub> = non-transgenic Dekalb® 6818C, G<sub>1</sub> glyphosate-tolerant Dekalb® 6818R, G<sub>2</sub> = glyphosate-tolerant and lepidopteran pest-resistant Dekalb® 6818S; Means of each parameter with different lowercase letters are significantly (p < 0.05) different.

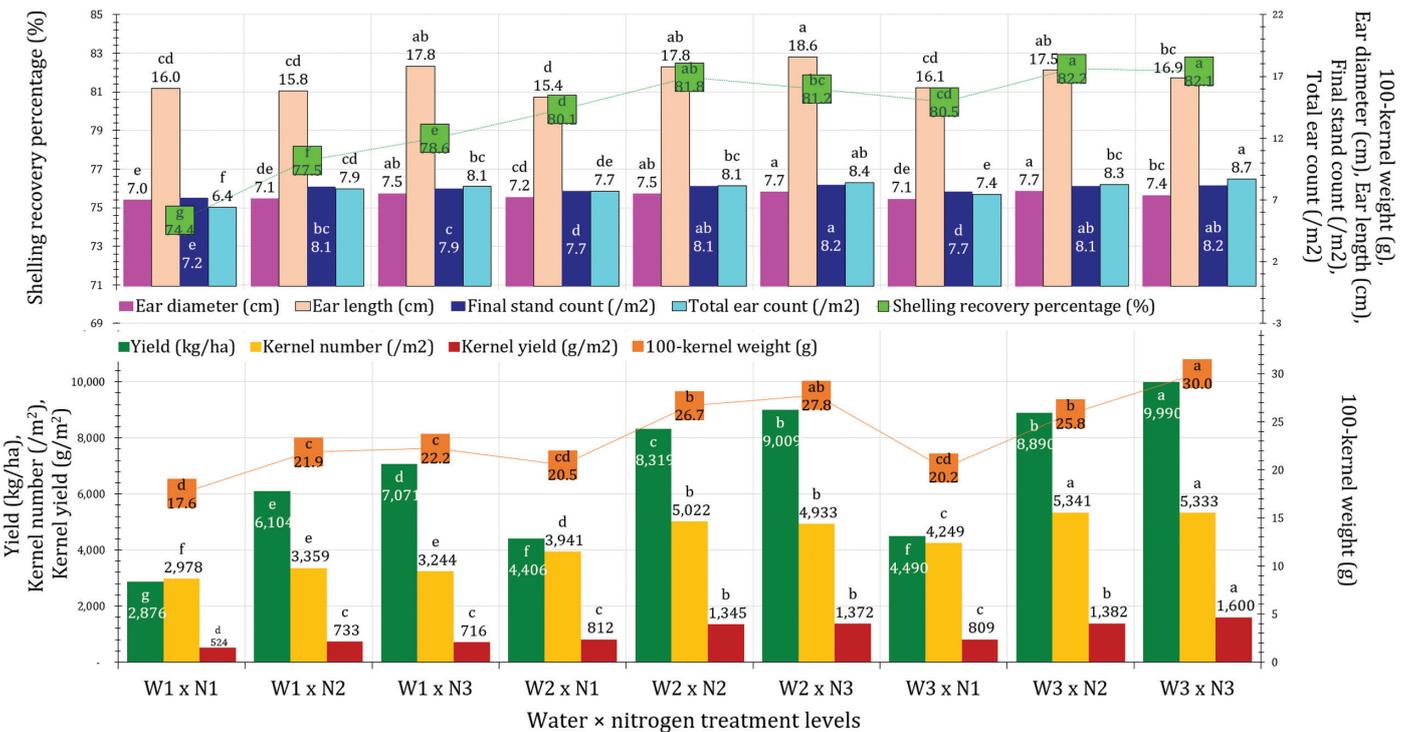
*Interaction effects of water (W) and nitrogen (N) on yield and yield components*

The interaction between W and N significantly impacted YLD and all components in Exp.1 (Fig.3), with the optimum conditions ( $W_3 \times N_3$ ) producing the highest YLD (10 t/ha), SP (82%), KN (5,337), KY (1.6 kg/m<sup>2</sup>), FSC (8.2 /m<sup>2</sup>) and  $KW_{100}$  (30 g). Against  $W_3 \times N_3$ , YLDs from the pre-anthesis drought plots— $W_2 \times N_3$ ,  $W_2 \times N_2$  and  $W_2 \times N_1$ —were lower by 10%, 17% and 56%, respectively. Under extreme drought, adequate N promoted YLD with the YLD values from  $N_3$  and  $N_2$  being higher by 60% and 52%, respectively, than  $N_1$ . Conversely, under no N, there was no significant difference in the YLD values between  $W_3$  and  $W_2$ , although both were still 55% higher than for  $W_1 \times N_1$ . The values of FSC, KN, KY, SP and TEC followed a similar trend as YLD—markedly increased under adequate W and N and lowest under  $W_1 \times N_1$  conditions (Fig. 3). Consistent with other research (Molla et al., 2014), the present study showed that adequate N under severe drought elevated plant development, with all N-applied plots having higher values for FSC (7%), KN (10%), KY (28%), SP (5%), and TEC (20%) than the no-N plots under prolonged drought. SP and KN were also significantly increased with increasing levels of W under fixed levels of N, showing that adequate soil

moisture enhances the plant response to N fertilizer, especially when a high N rate is applied (Mosser et al., 1998). A significant but more variable  $W \times N$  interaction was detected on ear size (Fig. 3); compared to plots where either or both W and N levels were adequate ( $W_2 \times N_3$ ,  $W_3 \times N_2$ ,  $W_2 \times N_2$ ,  $W_1 \times N_3$ ), there were reductions in ED (6%) and EL (12%) in plots where either or both were deficient ( $W_3 \times N_1$ ,  $W_1 \times N_2$ ,  $W_2 \times N_1$ ,  $W_1 \times N_1$ ), while for  $W_3 \times N_3$ , the values for ED and EL were in the middle of these two groups.

*Interaction effects of nitrogen (N) and isogenes (G) on yield and yield components*

The interaction between N and G markedly influenced  $KW_{100}$  and KY in both experiments, ED and EL in Exp.1 and KN and TEC in Exp.2 (Table 2). In general, all  $N \times G$  treatments recorded higher YLD,  $KW_{100}$  and KY values in Exp.2 than in Exp.1 (Fig. 4). In Exp.1, both  $G_0$  and  $G_1$  maximized  $KW_{100}$  and KY with 90 kg N/ha, with no real improvement with doubling the N rate; while for  $G_2$ , they were maximized at the highest N rate and declined with declining N. In Exp.2, both  $G_0$  and  $G_2$  produced the highest KY and KN under  $N_3$ , while  $G_1$  produced the same KY and KN values with only half the rate ( $N_2$ ).  $KW_{100}$  for all isogenes increased with increasing N in Exp.2. Significant  $N \times G$  interaction effects were



**Fig. 3** Interaction effects of water (W) and nitrogen (N) on yield (YLD), shelling percentage (SP), ear diameter (ED), ear length (EL), kernel number (KN), kernel weight ( $KW_{100}$ ), kernel yield (KY), final stand count (FSC) and total ear count (TEC).  $W_1$  = prolonged drought,  $W_2$  = pre-anthesis drought,  $W_3$  = well-irrigated,  $N_1$  = 0 kg N/ha,  $N_2$  = 90 kg N/ha,  $N_3$  = 180 kg N/ha; Means of each parameter with different lowercase letters are significantly ( $p < 0.05$ ) different.

also detected on ED and EL in Exp.1 (Fig.4). All G<sub>2</sub> plots (across N levels), along with N<sub>3</sub>×G<sub>0</sub> and N<sub>3</sub>×G<sub>1</sub>, produced the longest ears; while For EL and ED, G<sub>0</sub> followed an N<sub>3</sub> > N<sub>2</sub> > N<sub>1</sub> trend. The Ed values for G<sub>2</sub> and G<sub>1</sub> were maximized at N<sub>2</sub> and were not improved by additional N, but were reduced with decreasing N. In Exp.2, a significant mean separation for TEC was detected between all N–applied plots and no–N (N<sub>1</sub>) plots; while among the no–N (N<sub>1</sub>) plots, the TEC value for G<sub>2</sub> was 6% higher than for G<sub>1</sub>, but both were on par with G<sub>0</sub>.

Maize agrophysiological traits

Main effects of water (W), nitrogen (N) and isogenes (G) on agrophysiological traits

Water significantly influenced the total plant dry weight at anthesis (TPDW<sub>a</sub>) and at harvest (TPDW<sub>h</sub>), contribution of pre-anthesis dry matter to kernel (CPDMK), current assimilate transfer to kernel (CATK) and contribution of current assimilate to kernel (CCAK) in Exp.1 (Table 2). While TPDW<sub>a</sub> for W<sub>3</sub> was 8% and 11% higher than for W<sub>2</sub> and W<sub>1</sub>, respectively, the harvest biomass of W<sub>2</sub> and W<sub>3</sub> were on par and 8% higher than TPDW<sub>h</sub> of W<sub>1</sub>. Other studies have similarly observed plant recovery from pre-anthesis drought following rewatering (Moser et al., 1998; Molla et al., 2014). CPDMK for W<sub>1</sub> was 19% and 26% higher than for W<sub>3</sub> and W<sub>2</sub>, respectively (Table 2), which conformed with the tendency of CPDMK—the percentage of pre-anthesis dry matter reserve translocated to support kernel development when support from green leaves was insufficient (Molla et al., 2014)—to be higher in conditions

of stressed W and N. CATK is an important indicator of drought occurrence during grain development as it measures the assimilates transferred from green leaves to kernels (Boyer, 1996). Both W<sub>2</sub> and W<sub>3</sub> transferred 153% more assimilates to kernels, than W<sub>1</sub> (Table 2). CATK’s sensitivity to drought stress has led other researchers (Molla et al., 2014) to propose its use, along with KN, KW<sub>100</sub> and CCAK—the measure of translocated photosynthetic materials to sinks regardless of the sink size—as a breeding selection parameter for yield under drought conditions. For CCAK, W<sub>2</sub> was 7% and 26% higher than for W<sub>3</sub> and W<sub>1</sub>, respectively (Table 2). N also significantly impacted on all agrophysiological traits in both seasons (Table 2). Pre-anthesis and harvest biomass markedly increased with increasing N in both experiments. TPDW<sub>a</sub> for N<sub>3</sub> was 2% and 4% greater than for N<sub>2</sub> and N<sub>1</sub>, respectively, while the N–applied plots (N<sub>2</sub> and N<sub>3</sub>) produced 45% greater harvest biomass than N<sub>1</sub>. In both experiments, CPDMK for N<sub>1</sub> was 26% and 13% higher than for N<sub>2</sub> and N<sub>3</sub>, respectively. Likewise, CATK levels in the N–applied plots (N<sub>2</sub> and N<sub>3</sub>) in Exp.1 were on par and higher (123%) than for N<sub>1</sub>. In Exp.2, the significant positive effect of N on CATK was more pronounced, being 13% and 141% higher for N<sub>3</sub> than for N<sub>2</sub> and N<sub>1</sub>, respectively. In both experiments, the values of CCAK for N<sub>2</sub> and N<sub>3</sub> were on par and higher than for N<sub>1</sub>. There were significant isogene differences for TPDW<sub>a</sub> in both experiments (Table 2) but there was a small but significant increase in TPDW<sub>h</sub> for G<sub>2</sub> versus both G<sub>0</sub> and G<sub>1</sub> in both experiments. This may have been due to residual genetic variation arising from the multiple breeding steps to achieve near-isogenic commercial hybrids (Venkatesh et al., 2015). G had no significant impacts on CPDMK, CATK and CCAK in both experiments (Table 2).

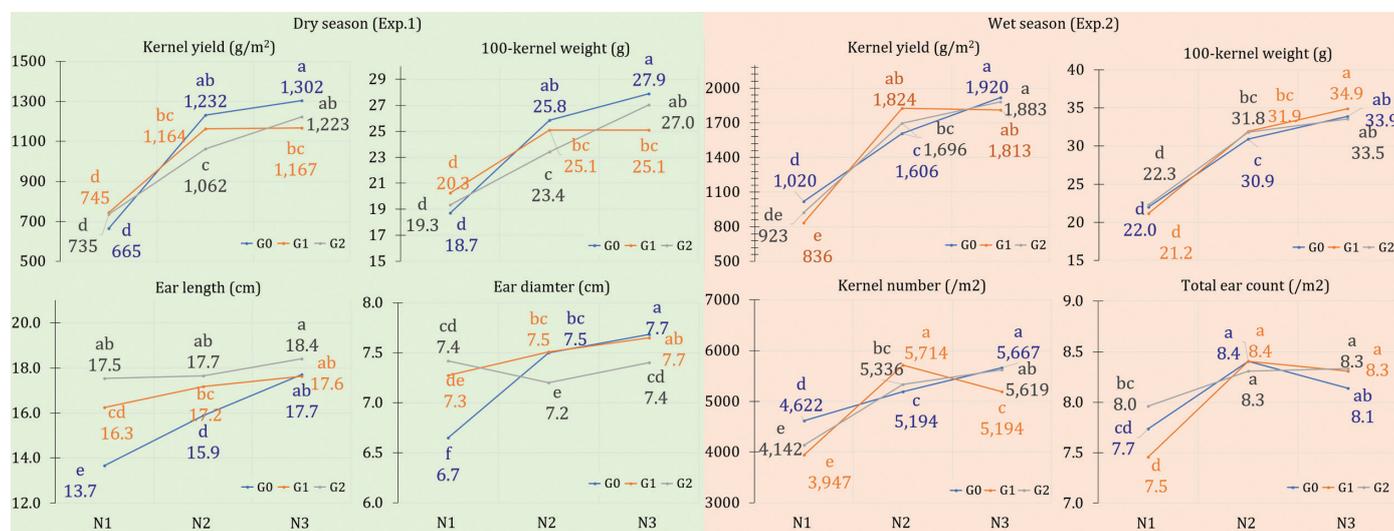


Fig. 4 Interaction effects of nitrogen (N) and isogenes (G) on kernel yield (KY), kernel weight (KW<sub>100</sub>), kernel number (KN), total ear count (TEC), ear length (EL) and ear diameter (ED) in 2016 dry (Exp.1) and wet (Exp.2) seasons, where N<sub>1</sub> = 0 kg N/ha, N<sub>2</sub> = 90 kg N/ha, N<sub>3</sub> = 180 kg N/ha, G<sub>0</sub> = non-transgenic Dekalb® 6818C, G<sub>1</sub> glyphosate-tolerant Dekalb® 6818R, G<sub>2</sub> = glyphosate-tolerant and lepidopteran pest-resistant Dekalb® 6818S; Means of each parameter in the same experiment with different lowercase letters are significantly (p < 0.05) different.

**Table 2** Main and interaction effects of water (W), nitrogen (N) and isogenes (G) on total plant dry weight at anthesis (TPDW<sub>a</sub>), total plant dry weight at harvest (TPDW<sub>h</sub>), contribution of pre-anthesis dry matter to kernel (CPDMK), current assimilate transfer to kernel (CATK) and contribution of current assimilate to kernel (CCAK) in 2016 dry (Exp.1) and wet (Exp.2) seasons

Treatment	TPDW <sub>a</sub> (g/m <sup>2</sup> )	TPDW <sub>h</sub> (g/m <sup>2</sup> )	CPDMK (%)	CATK (g/m <sup>2</sup> )	CCAK (%)
<i>Main Effects</i>					
Exp.1					
Water (W)	**	**	**	**	**
W <sub>1</sub>	1,848 <sup>c</sup>	1,551 <sup>b</sup>	48.66 <sup>a</sup>	361 <sup>b</sup>	51.34 <sup>c</sup>
W <sub>2</sub>	1,904 <sup>b</sup>	1,663 <sup>a</sup>	22.61 <sup>c</sup>	936 <sup>a</sup>	77.39 <sup>a</sup>
W <sub>3</sub>	2,046 <sup>a</sup>	1,677 <sup>a</sup>	30.01 <sup>b</sup>	894 <sup>a</sup>	69.99 <sup>b</sup>
Nitrogen (N)	**	**	**	**	**
N <sub>1</sub>	1,571 <sup>c</sup>	1,255 <sup>b</sup>	48.80 <sup>a</sup>	401 <sup>b</sup>	51.20 <sup>b</sup>
N <sub>2</sub>	2,092 <sup>b</sup>	1,822 <sup>a</sup>	25.40 <sup>b</sup>	881 <sup>a</sup>	74.60 <sup>a</sup>
N <sub>3</sub>	2,134 <sup>a</sup>	1,814 <sup>a</sup>	27.08 <sup>b</sup>	909 <sup>a</sup>	72.92 <sup>a</sup>
Isogenes (G)	ns	**	ns	ns	ns
G <sub>0</sub>	1,924	1,621 <sup>b</sup>	34.24	764	65.76
G <sub>1</sub>	1,927	1,618 <sup>b</sup>	34.12	715	65.88
G <sub>2</sub>	1,945	1,651 <sup>a</sup>	32.92	712	67.08
Exp.2					
Nitrogen (N)	**	**	**	**	**
N <sub>1</sub>	1,797 <sup>c</sup>	1,536 <sup>c</sup>	28.63 <sup>a</sup>	665 <sup>c</sup>	71.37 <sup>b</sup>
N <sub>2</sub>	2,196 <sup>b</sup>	1,910 <sup>b</sup>	16.85 <sup>b</sup>	1,418 <sup>b</sup>	83.15 <sup>a</sup>
N <sub>3</sub>	2,261 <sup>a</sup>	1,990 <sup>a</sup>	14.43 <sup>b</sup>	1,605 <sup>a</sup>	85.57 <sup>a</sup>
Isogenes (G)	ns	**	ns	ns	ns
G <sub>0</sub>	2,077	1,784 <sup>b</sup>	20.36	1,222	79.64
G <sub>1</sub>	2,071	1,807 <sup>b</sup>	20.59	1,226	79.41
G <sub>2</sub>	2,106	1,845 <sup>a</sup>	18.96	1,240	81.04
<i>Interaction Effects</i>					
W × N × G	**	**	**	**	**
W × N	**	**	**	**	**
W × G	**	**	ns	ns	ns
N × G (Exp.1)	**	**	**	**	**
N × G (Exp.2)	**	**	**	*	**

W<sub>1</sub> = prolonged drought, W<sub>2</sub> = pre-anthesis drought, W<sub>3</sub> = well-irrigated, N<sub>1</sub> = 0 kg N/ha, N<sub>2</sub> = 90 kg N/ha, N<sub>3</sub> = 180 kg N/ha, G<sub>0</sub> = non-transgenic Dekalb<sup>®</sup> 6818C, G<sub>1</sub> glyphosate-tolerant Dekalb<sup>®</sup> 6818R, G<sub>2</sub> = glyphosate-tolerant and lepidopteran pest-resistant Dekalb<sup>®</sup> 6818S;

\*, \*\* denote significance ( $p < 0.05$ ) and high significance ( $p < 0.01$ ), respectively; ns = non-significance ( $p > 0.05$ ). Means in a column within each main effect superscripted with different lowercase letters are significantly ( $p < 0.05$ ) different.

#### *Interaction effects of water (W), nitrogen (N) and isogenes (G) on agrophysiological traits*

The interaction of W, N and G markedly influenced TPDW<sub>a</sub>, TPDW<sub>h</sub>, CPDMK, CATK and CCAK (Fig. 5). There was a more prominent role of G in the three-way interaction to impact the agrophysiological traits when both W and N were at optimum levels, when both were in extreme deficit and when N was not applied. Under optimum levels (W<sub>3</sub> and N<sub>3</sub>), TPDW<sub>a</sub> for G<sub>2</sub> was highest across all treatments, and 4% higher than for both G<sub>1</sub> and G<sub>0</sub>; CPDMK for G<sub>0</sub> was 10% lower than for G<sub>2</sub> and G<sub>1</sub>, while CATK for G<sub>0</sub> the was highest overall and 18% higher than for G<sub>2</sub> and G<sub>1</sub>. Under extreme stress (W<sub>1</sub> and N<sub>1</sub>), G<sub>1</sub> produced the lowest TPDW<sub>a</sub>—7% lower than for both G<sub>2</sub> and G<sub>0</sub>; and the lowest TPDW<sub>h</sub>—6% lower than for both

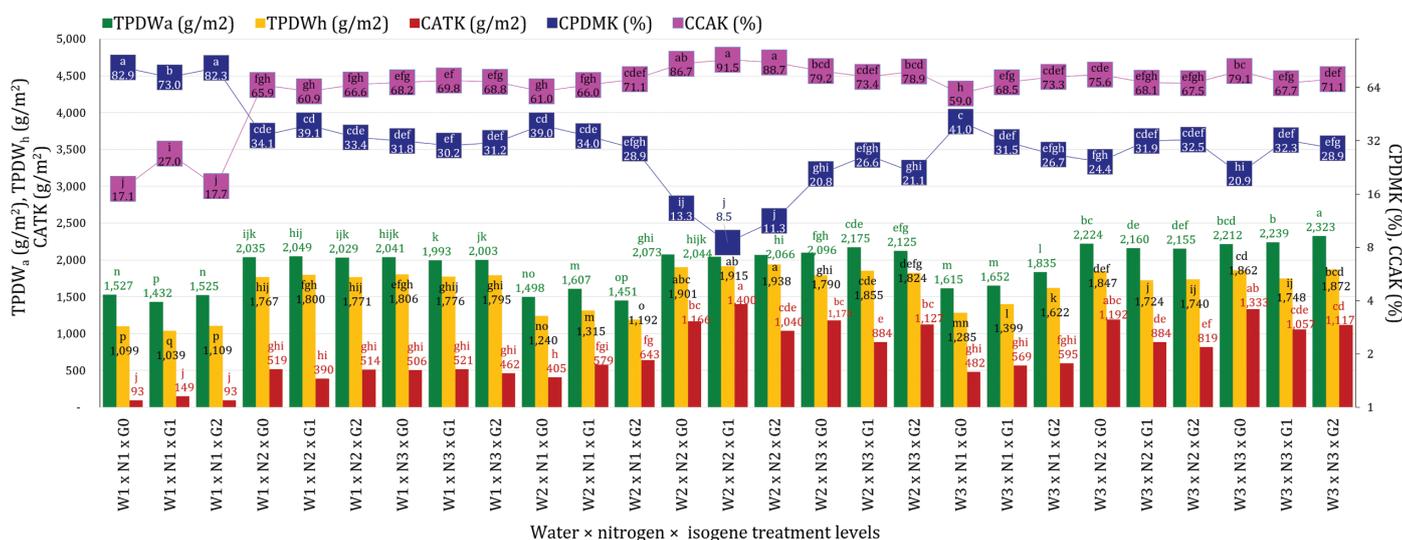
G<sub>2</sub> and G<sub>0</sub>. Under the same severe conditions (W<sub>1</sub> and N<sub>1</sub>), the G<sub>0</sub> and G<sub>2</sub> plots recorded the highest overall CPDMK (83%)—10% higher than for G<sub>1</sub>. In the absence of N (N<sub>1</sub>), G<sub>2</sub> produced the highest TPDW<sub>a</sub> among the well irrigated plots (W<sub>3</sub>), while in the absence of N under W<sub>2</sub> conditions, TPDW<sub>a</sub> for G<sub>1</sub> was highest among the pre-anthesis drought plots. Significant CPDMK means separation among isogenes was also detected under no-N conditions; in the fully irrigated conditions (W<sub>3</sub>), CPDMK for G<sub>0</sub> was 12% higher than for both G<sub>1</sub> and G<sub>2</sub>; in the pre-anthesis drought (W<sub>2</sub>) conditions, the CPDMK values for G<sub>0</sub> and G<sub>1</sub> were 9% more than for G<sub>2</sub>. At harvest (TPDW<sub>h</sub>), the highest biomass (1.9 kg/m<sup>2</sup>) was achieved under moderate W and N conditions. These same plots with the highest TPDW<sub>h</sub> also recorded the lowest

CPDMK values (in the range 8.5–13.3%). Under the same moderate W and N conditions ( $W_2$  and  $N_2$ ),  $G_1$  produced the highest CATK—20% and 35% higher than for  $G_0$  and  $G_2$ , respectively. However, all three treatments had equal highest CCAK values (in the range 87–92%).

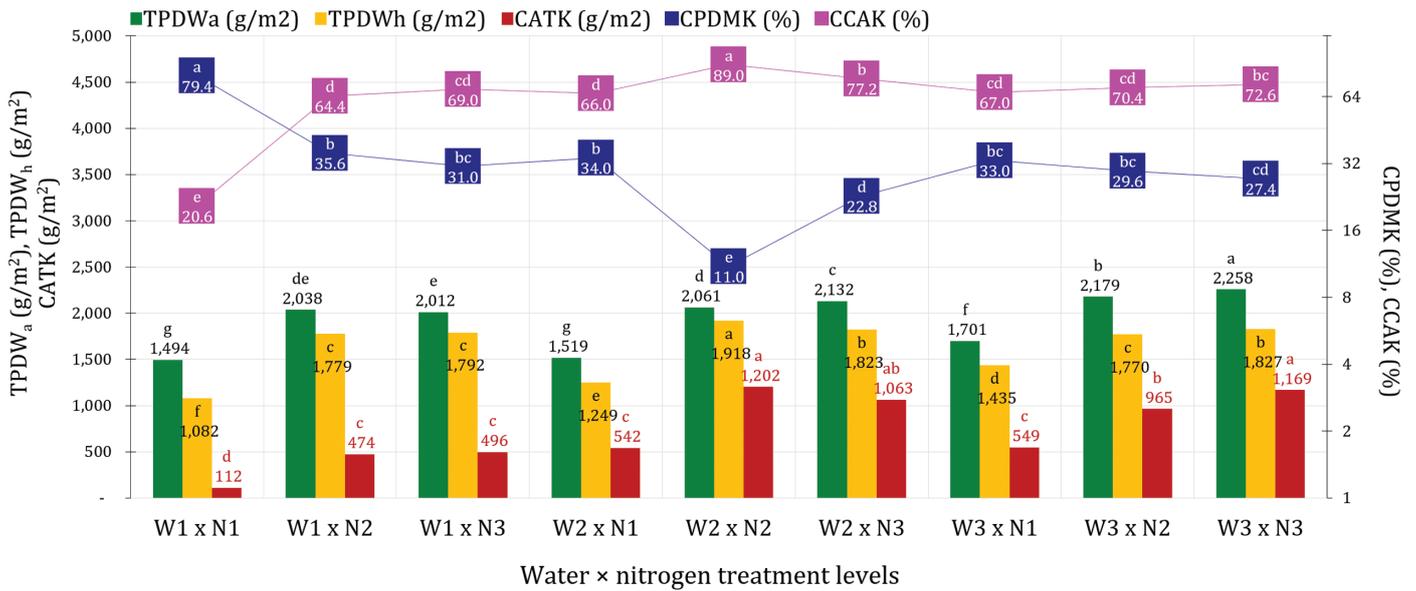
*Interaction effects of water (W) and nitrogen (N) on agrophysiological traits*

The interaction of W and N significantly influenced  $TPDW_a$ ,  $TPDW_h$ , CPDMK, CATK, and CCAK (Fig. 6).  $TPDW_a$  notably increased with increasing W and N, with  $W_3 \times N_3$  being the highest overall at 9% higher than all the N-applied plots subjected to drought treatments [ $(W_1, W_2) \times (N_2, N_3)$ ], and 35% higher than the no-N, drought-stressed plots ( $W_1 \times N_1$  and  $W_2 \times N_1$ ). Other studies (Moser et al., 1998; Kebede et al., 2014) reported a low pre-anthesis biomass in drought-stressed plants was the net effect of a series of events starting with a significant reduction in the size or number or both of vascular bundles in the ear peduncle, which reduced the ability of the xylem to transport water and nutrients from the soil to the plant, thereby reducing the plant's ability to transport assimilates and, subsequently, impairing organ development. At harvest,  $W_2 \times N_2$  produced the highest  $TPDW_h$ —5% significantly higher than for both  $W_3 \times N_3$  and  $W_2 \times N_3$ —and the lowest CPDMK. This was not different from what Molla et al. (2014) observed in

N-sufficient, moderately drought-stressed plots achieving high YLD values and harvest biomass and low CPDMK values. For CCAK,  $W_2 \times N_2$  (89%) was the highest, followed by [ $(W_2, W_3) \times N_3$ ] at 75%. Under no-N, water levels played a more prominent role in impacting harvest biomass, following a significant  $W_3 > W_2 > W_1$  trend. Plots under severely restricted water and N conditions ( $W_1$  and  $N_1$ ) produced the highest CPDMK (79%), and the lowest CATK (112 g/m<sup>2</sup>) and CCAK (21%). CPDMK was reduced (27%) when either W or N was deficient, and the other was adequate: [ $N_1 \times (W_2, W_3)$ ] and [ $W_1 \times (N_2, N_3)$ ]. There was a simultaneous gain in  $TPDW_h$  but a lack of translocation (as CPDMK) in pre-anthesis drought plots, although Boyer (1996) suggested that these parameters needed to be measured multiple times within the season due to the transient nature of the dry matter transport system. The highest CATK was observed in the high-input plots ( $W_3 \times N_3, W_2 \times N_2, W_2 \times N_3$ ) but no significant differences were detected when one factor was severely deficient and the other factor was optimal, such as [ $N_1 \times (W_2, W_3)$ ] and [ $W_1 \times (N_2, N_3)$ ].  $N_2$  plots under moderate drought conditions ( $W_2$ ) had the lowest CPDMK and the highest CATK, CCAK and  $TPDW_h$  values, suggesting both a drought-induced slowdown of early grain development—demanding less translocation of materials—and the ability of moderately desiccated plants to support grain fill with timely rewatering.



**Fig. 5** Interaction effects of water (W), nitrogen (N) and isogenes (G) on total plant dry weight at anthesis ( $TPDW_a$ , g/m<sup>2</sup>) and at harvest ( $TPDW_h$ , g/m<sup>2</sup>), contribution of pre-anthesis dry matter to kernel (CPDMK, %), current assimilate transfer to kernel (CATK, g/m<sup>2</sup>) and contribution of current assimilate to kernel (CCAK, %) in 2016 dry season (Exp.1), where  $W_1$  = prolonged drought,  $W_2$  = pre-anthesis drought,  $W_3$  = well-irrigated,  $N_1$  = 0 kg N/ha,  $N_2$  = 90 kg N/ha,  $N_3$  = 180 kg N/ha,  $G_0$  = non-transgenic Dekalb® 6818C,  $G_1$  glyphosate-tolerant Dekalb® 6818R,  $G_2$  = glyphosate-tolerant and lepidopteran pest-resistant Dekalb® 6818S; Means of each parameter with different lowercase letters are significantly ( $p < 0.05$ ) different.



**Fig. 6** Interaction effects of water (W) and nitrogen (N) on total plant dry weight at anthesis (TPDW<sub>a</sub>, g/m<sup>2</sup>), total plant dry weight at harvest (TPDW<sub>h</sub>, g/m<sup>2</sup>), contribution of pre-anthesis dry matter to kernel (CPDMK, %), current assimilate transfer to kernel (CATK, g/m<sup>2</sup>) and contribution of current assimilate to kernel (CCAK, %) in 2016 dry season (Exp.1), where W<sub>1</sub> = prolonged drought, W<sub>2</sub> = pre-anthesis drought, W<sub>3</sub> = well-irrigated, N<sub>1</sub> = 0 kg N/ha, N<sub>2</sub> = 90 kg N/ha, N<sub>3</sub> = 180 kg N/ha;

Means of each parameter with different lowercase letters are significantly ( $p < 0.05$ ) different.

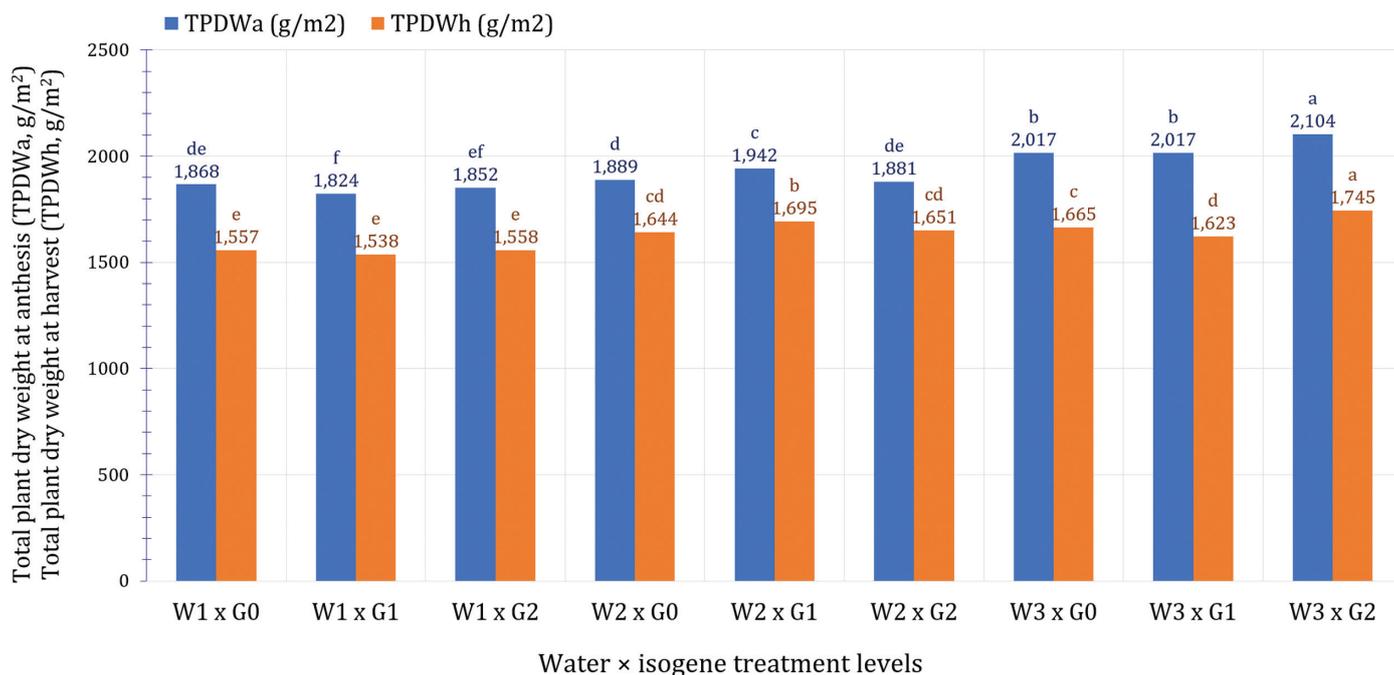
#### Interaction effects of water (W) and isogenes (G) on agrophysiological traits

The interaction of W and G was strongly influenced by W to impact TPDW<sub>a</sub>, with treatments following a W<sub>3</sub> > W<sub>2</sub> > W<sub>1</sub> trend (Fig. 7). When W was not severely deficient, isogene played a more prominent role in interacting with W to impact pre-anthesis biomass. W<sub>3</sub> × G<sub>2</sub> produced the highest overall TPDW<sub>a</sub> (2.10 kg/m<sup>2</sup>)—4% higher than for both W<sub>3</sub> × G<sub>1</sub> and W<sub>3</sub> × G<sub>0</sub>, and 11% higher than for W<sub>2</sub> × G<sub>2</sub>. This demonstrated G<sub>2</sub>'s ability to accumulate higher vegetative biomass under adequate irrigation, which ultimately translated to the highest harvest biomass; under full irrigation (W<sub>3</sub>), TPDW<sub>h</sub> for G<sub>2</sub> was 5% and 8% higher than for G<sub>0</sub> and G<sub>1</sub>, respectively (Fig. 7). Under pre-anthesis drought (W<sub>2</sub>), TPDW<sub>h</sub> for G<sub>1</sub> was 3% significantly higher than for both G<sub>2</sub> and G<sub>0</sub>. No significant differences were detected among isogenes for severe drought conditions (W<sub>1</sub>), which produced the lowest overall TPDW<sub>h</sub>s (1.6 kg/m<sup>2</sup>).

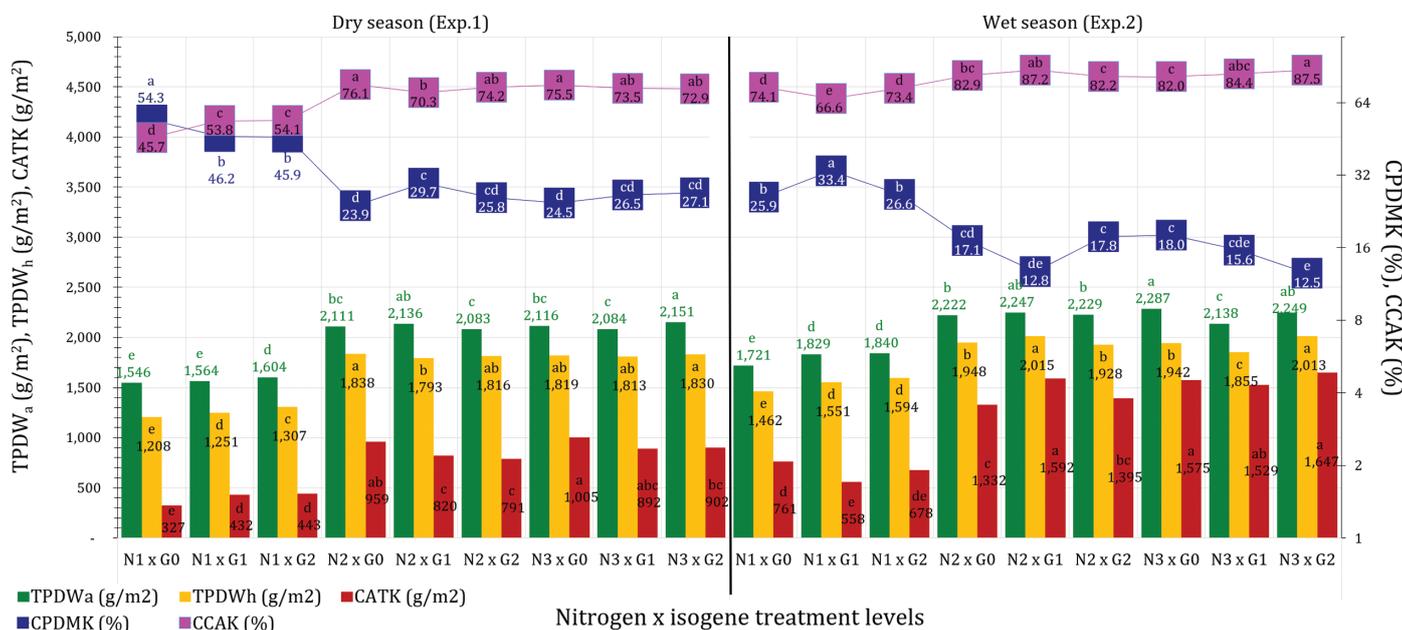
#### Interaction effects of nitrogen (N) and isogenes (G) on agrophysiological traits

The interaction between N and G significantly impacted all agrophysiological traits in both experiments (Table 2). N played a stronger role in interacting with G to influence both biomass parameters; all N-applied plots summarily

produced higher TPDW<sub>a</sub>s and TPDW<sub>h</sub>s than the no-N plots in both seasons (Fig. 8). G played a prominent role when N was either optimum or severely deficient. In Exp.1, at the highest N rate (N<sub>3</sub>), for TPDW<sub>a</sub>, G<sub>2</sub> was 2% higher than for G<sub>0</sub> and on par with G<sub>1</sub>, while in Exp.2, both G<sub>2</sub> and G<sub>0</sub> values for TPDW<sub>a</sub> were highest overall and 4% higher than for G<sub>1</sub>. N<sub>3</sub> × G<sub>2</sub> produced the highest harvest biomass in Exp.1 [along with N<sub>3</sub> × (G<sub>0</sub>, G<sub>1</sub>) and N<sub>2</sub> × (G<sub>1</sub>, G<sub>2</sub>)] and in Exp.2 (along with N<sub>2</sub> × G<sub>1</sub>). Under no N (N<sub>1</sub>), TPDW<sub>a</sub> for G<sub>2</sub> was still 2% and 5% higher than for G<sub>1</sub> and G<sub>0</sub>, respectively, in Exp.1 and 9% higher than for G<sub>0</sub> in Exp.2. This result demonstrated a more efficient biomass production of G<sub>2</sub> under N deficiency. All N-deprived (N<sub>1</sub>) plots recorded 23% (Exp.1) and 13% (Exp.2) higher values for CPDMK than for all the N-applied (N<sub>2</sub> and N<sub>3</sub>) plots. Significant CPDMK differences among isogenes were detected under no-N plots but not in N-applied plots. Across isogenes, N-applied plots had the highest CCAK values (0.95–1.15 kg/m<sup>2</sup>), while all the N-starved plots, regardless of the isogene, had the lowest CCAK values (0.47 kg/m<sup>2</sup>). The CATK value for all isogenes under N<sub>3</sub> averaged 1.08 kg/m<sup>2</sup>. Under N<sub>2</sub>, both G<sub>0</sub> and G<sub>1</sub> had 11% higher CATK values than G<sub>2</sub>. All N-applied plots recorded 76% CCAK—19% and 24% higher than for [N<sub>1</sub> × (G<sub>1</sub>, G<sub>2</sub>)] and N<sub>1</sub> × G<sub>0</sub>, respectively.



**Fig. 7** Interaction effects of water (W) and isogenes (G) on total plant dry weight at anthesis (TPDW<sub>a</sub>) and total plant dry weight at harvest (TPDW<sub>h</sub>) in 2016 dry season (Exp.1), where W<sub>1</sub> = prolonged drought, W<sub>2</sub> = pre-anthesis drought, W<sub>3</sub> = well-irrigated, G<sub>0</sub> = non-transgenic Dekalb® 6818C, G<sub>1</sub> = glyphosate-tolerant Dekalb® 6818R, G<sub>2</sub> = glyphosate-tolerant and lepidopteran pest-resistant Dekalb® 6818S; Means of each parameter with different lowercase letters are significantly ( $p < 0.05$ ) different.



**Fig. 8** Interaction effects of nitrogen (N) and isogenes (G) on total plant dry weight at anthesis (TPDW<sub>a</sub>, g/m<sup>2</sup>) and at harvest (TPDW<sub>h</sub>, g/m<sup>2</sup>), contribution of pre-anthesis dry matter to kernel (CPDMK, %) and current assimilate transfer to kernel (CATK, g/m<sup>2</sup>) and contribution of current assimilate to kernel (CCAK, %) in 2016 dry (Exp.1) and wet (Exp.2) seasons, where N<sub>1</sub> = 0 kg N/ha, N<sub>2</sub> = 90 kg N/ha, N<sub>3</sub> = 180 kg N/ha, G<sub>0</sub> = non-transgenic Dekalb® 6818C, G<sub>1</sub> = glyphosate-tolerant Dekalb® 6818R, G<sub>2</sub> = glyphosate-tolerant and lepidopteran pest-resistant Dekalb® 6818S; Means of each parameter in the same experiment with different lowercase letters are significantly ( $p < 0.05$ ) different.

In conclusion, a hybrid's yield and agrophysiological response were markedly influenced by water, N and their synergistic interaction, especially at optimum levels or when either one was in extreme deficit. Ample soil moisture and N produced the highest yields and harvest biomass in both seasons. Under short (pre-anthesis) and prolonged (bracketing flowering) drought conditions, sufficient soil N aided in plant recovery and yield production, while under low to no N, the higher soil moisture improved yield and plant response to N fertilization. The dry season study confirmed the equivalence in yield and response of the GM hybrids—G<sub>1</sub> (herbicide-tolerant Dekalb® 6818R, with transformation event, NK603) and G<sub>2</sub> (herbicide-tolerant and insect-resistant Dekalb® 6818S, with MON89034 × NK603 events stack)—to their non-GM counterparts, G<sub>0</sub> (Dekalb® 6818C) under negligible target insect pressure. Hybrid played a more prominent role in interacting with W and N to impact pre-anthesis and harvest biomass when both W and N were optimal or when N was deficient but W was ample. G<sub>2</sub> also produced significantly higher yields than G<sub>1</sub> and G<sub>0</sub> under concurrent moderate drought and severe N deficiency and under severe drought with sufficient N. While the observed advantages of G<sub>2</sub> over its counterparts were statistically significant, they were also biologically small, which could be attributed to residual genetic variation associated with conventional breeding for near-isogenic hybrids. For the wet season, the 6% yield advantage of both GM hybrids over the non-GM hybrid could be ascribed to the benefits of a convenient and effective weed management system (spraying glyphosate over herbicide-tolerant corn) over manual weeding operations in non-GM crops. Under no N, no yield differences were observed among hybrids; however, isogene and its interaction with the N rate influenced responsiveness to N availability to maximize yields across the two seasons. Both G<sub>2</sub> and G<sub>0</sub> required 180 kg N/ha to achieve their top yields in the dry season, but only 90 kg N/ha in the wet season, while G<sub>1</sub> reached its optimum YLD value with 90 kg N/ha in both seasons. While the evolutionary trade-off between high yield and adaptability to low N is commonplace in new hybrids—which have been selected under (and for) high N-fertilizer input—the present results showed that these three isogenes could maintain high grain yield under conditions of moderate N supply. This study also supported that under low water and N conditions, hybrids which can store and effectively transport large amounts of pre-anthesis assimilates into developing kernels have higher grain yield potential. Kernel number and weight, pre-anthesis biomass, current assimilate transfer to kernel (CATK) and contribution of current assimilate

to kernel (CCAK) were strongly positively correlated to yield and could be used as important screening parameters for yield under water and N deficient conditions.

### Conflict of Interest

The authors declare that there are no conflicts of interest.

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### References

- Boyer, J.S. 1996. Advances in drought tolerance in plants. *Adv. Agron.* 56: 187–219.
- The International Service for the Acquisition of Agri-biotech Applications. 2018. Pocket K No. 56: Substantial Equivalence of GM and Non-GM Crops. Los Banos, Laguna, [https:// www.isaaa.org/resources/publications/](https://www.isaaa.org/resources/publications/), 20 December 2016.
- Kebede, H., Sui, R., Fisher, D., Reddy, K., Bellaloui, N., Molin, W. 2014. Corn yield response to reduced water use at different growth stages. *Agric. Sci.* 5: 1305–1315.
- Laurena, A.C. 2005. Feed safety of agricultural plant products derived through biotechnology. In: *Science-based Reviews: Issues and Concerns of Modern Biotechnology*. FAST-PSHC Foundation. Manila, the Philippines, pp. 59–91.
- Molla, S., Nakasathien, S., Sarobol, E., Vichukit, V. 2014. Effects of nitrogen and water on maize varieties under short and prolonged drought. *J. Dev. Sustain. Agric.* 9: 97–110.
- Moser, S., Feil, B., Stamp, P., Thiraporn, R. 1998. Tropical maize under pre-anthesis drought and low nitrogen supply. *Developing Drought and Low N Tolerant Maize*. In: *Proceedings of a Symposium CIMMYT Sustainable Maize and Wheat Systems for the Poor*, El Batan, Mexico, pp. 159–162.
- Pandey, R., Maranville, J., Admou, A. 2000. Deficit irrigation and nitrogen effects on maize in a Sahelian environment: I. grain yield and yield components. *Agric. Water Manag.* 46: 1–13.
- Singletary, G.W., Below, F.E. 1990. Nitrogen-induced changes in the growth and metabolism of developing maize kernels grown *in vitro*. *Plant Physiol.* 92: 160–167.
- Venkatesh, T.V., Cook, K., Liu, B., Perez, T., Wise, A., Tihich, R., Feng, P., Harrigan, G.G. 2015. Compositional differences between near-isogenic GM and conventional maize hybrids are associated with backcrossing practices in conventional breeding. *Plant Biotechnol. J.* 13: 200–210.