

Effects of Nitrogen and Water on Maize Varieties under Short and Prolonged Drought

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Inadequate management of nitrogen and water stress are the main factors underlying poor growth and yield in maize (*Zea mays* L.). To evaluate the effects of nitrogen and water stress on the growth and physiology of maize plants, two field experiments were conducted at the National Corn and Sorghum Research Center, Thailand, during 2010–2011 and 2012 under short pre-anthesis drought and prolonged flowering-stage drought, respectively. A split-plot design with factorial randomized complete block arrangement was used for the experiment, with two water regimes (well-watered and water-stressed) forming the main plots and two maize hybrids (Pioneer 30B80 and Suwan 4452) and three nitrogen levels (0, 160 [optimal] and 320 [supra-optimal] kg N ha⁻¹) forming the subplots. We found that supra-optimal nitrogen (urea) temporarily changed the soil pH significantly. Optimal nitrogen and well-watered conditions enhanced the net assimilation rate at the vegetative stage (NAR_v), ovule number per primary ear (ON_{pe}), leaf area duration in the reproductive period (LAD_r), specific leaf weight at anthesis (SLW_a), current assimilate transfer to kernels (CATK), chlorophyll content at anthesis (Chl_a), nitrogen use efficiency, biomass yield, and kernel yield per plant (KYP). In contrast, supra-optimal and zero nitrogen and water stress had detrimental effects on these parameters, except Chl_a. Prolonged flowering-stage drought was found to be more detrimental than short pre-anthesis drought regarding plant performance. Greater root-to-shoot ratio, LAD_r, Chl_a, nitrogen use efficiency, NAR_v, and ON_{pe} were recorded as traits of nitrogen and water-stress tolerance in Pioneer 30B80. The optimal nitrogen level improved drought resistance, especially under short pre-anthesis drought. The correlations between KYP and LAD_r, Chl_a, NAR_v, ON_{pe}, and CATK were positive and highly significant, and NAR_v, ON_{pe}, and CATK had significant and positive direct effects on KYP under both short pre-anthesis and prolonged flowering-stage drought.

Key words: nitrogen, biomass yield, maize, soil pH, water stress

Introduction

With rapid changes in global climate, drought and food security are of great concern. Controlling atmospheric carbon levels through carbon sequestration by increased total plant biomass, especially greater root biomass, is a promising option for reducing global warming (Ontl and Schulte, 2012). As a C₄ plant, maize is a promising candidate for such purposes (Wang *et al.*, 2012). Nitrogen helps maize plants to increase leaf area, which enhances photosynthesis and consequently grain yield and total biomass (Westgate *et al.*, 2004).

Sustainable crop production depends on good farming practices (Ransom, 2004), with water and nitrogen fertilizer management being the most important factors. Studies have shown that nitrogen plays an important role in plant growth and yield during drought (e.g., Waraich *et al.*, 2011). Nevertheless, excess nitrogen fertilizer application is a common problem in many areas (Zhao *et al.*, 2006) and may have a detrimental effect on crops and the environment (Clemens *et al.*, 2008). Drought is the greatest constraint affecting maize yields in developing countries (Ekasingh *et al.*, 2004). The effects of water stress may depend as much on the duration and timing of the stress as on

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the level of stress imposed.

Increased abiotic stress tolerance is related to increased maize yield and yield stability, which are influenced by the number of ears and kernels (Tollenaar and Wu, 1999). Some maize varieties have constitutive stress tolerance mechanisms that may be associated with the establishment of reproductive structures (sink size). When sufficient water, nitrogen, and other nutrients are available, maize plants may set more ovules during ear initiation in the vegetative stage. However, maize produces many more ovules than those that survive to maturity (Tollenaar and Wu, 1999), and abortion occurs when the ovules fail to extrude silk because of slow growth rates (Westgate and Bassetti, 1991). These findings suggest that a higher net assimilation rate at the vegetative stage (NAR_v), especially the pre-flowering stage, is very important for optimum growth in maize. Increased drought tolerance can reduce kernel and ear abortion under drought conditions (Edmeades *et al.*, 1993), which mostly depends on a sufficient current assimilate supply during the grain-filling stage (Tollenaar and Wu, 1999), and greater leaf longevity likely influences current assimilate production. Nitrogen concentrations in the soil and plant xylem sap can vary in response to changes in the amount and form of nitrogen in the soil (Peuke *et al.*, 1996), but what was the effects on soil pH, which may be very important for nutrient availability and plant signaling, is not clearly understood. The physiological mechanisms underlying tolerance to specific abiotic stress factors have been investigated in several genetic improvement studies of tropical maize (Westgate and Bassetti, 1991; Edmeades *et al.*, 1993; Muchow, 1998; Tollenaar and Wu, 1999). Nevertheless, little is known about the physiological tolerance mechanisms that contribute to increased grain and biomass yields under simultaneous nitrogen and drought stress, the detrimental effects of which must be minimized to achieve food security.

Special characteristics of crops—adaptive changes associated with drought tolerance and sustained under nitrogen stress—may be indicative of constitutive stress tolerance mechanisms in maize that contribute to increased yield and yield stability. To improve our understanding of those mechanisms, it is important to study the positive and negative effects of nitrogen on maize under short and prolonged drought.

Materials and Methods

Two field studies were conducted at the National Corn and Sorghum Research Center, Nakhon Ratchasima, Thailand, during the regular dry period in 2010–2011 (hereafter referred as 2011) and 2012. The soil at the study site was clayey, had a pH level of 6.8, and contained medium levels of organic matter and medium to high levels of phosphorus, potassium, calcium, and magnesium. There was no rainfall in 2011 during the water-stress period, but small rainfall events occurred twice in 2012, amounting to 2 and 3 mm at 12 and 21 days after water withholding, respectively. The mean daily temperature was 24.1°C in 2011 and 27.0°C in 2012.

The experiments had a split-plot design with factorial arrangements of the treatments in three replicates. Two water regimes (well-watered [control] and water-stressed) were assigned to the main plots, and combinations of two varieties (Pioneer 30B80 and Suwan 4452) and three nitrogen levels (0 kg N ha⁻¹ [zero], 160 kg N ha⁻¹ [optimal and control], and 320 kg N ha⁻¹ [supra-optimal]) were randomly assigned in the subplots. We defined the optimal level of nitrogen based on Moser *et al.* (2006).

Seeds were planted on 3 December 2010 and 14 January 2012, with 75 cm × 25 cm spacing and one plant per hill. In each treatment, half the nitrogen as urea (46% N) was applied as a basal application and the other half was applied as a side dressing at 34 days after planting, at about the eight-leaf stage. Prior to this stage, sprinkler irrigation was used, and thereafter furrow irrigation was provided on a weekly basis. A tensiometer was used to monitor the soil water tension and to ensure field capacity. In the water-stressed treatment, irrigation was suspended from 35 to 64 days after planting under the short pre-anthesis drought in 2011 and from 35 to 76 days after planting under the prolonged flowering-stage drought in 2012; thereafter, irrigation was resumed until physiological maturity, at levels equal to those in the well-watered block.

To determine the total dry matter, including roots (collected from 0 to 40 cm soil depth), each week during the experimental period five plants were chosen randomly from each subplot. Dry matter of different plant parts was determined after oven-drying the material until it reached constant weight. Green leaf area (length × maximum width × 0.75) was also measured weekly during the vegetative stage (water-stress pe-

riod), anthesis stage, and grain-filling period until physiological maturity.

The NAR_v during the water-stress period and leaf area duration during both the vegetative (LAD_v) and reproductive (LAD_r) stages were estimated by using the equations of Hunt (1978):

$$NAR_v \text{ (g m}^{-2} \text{ d}^{-1}\text{)} = TDM/LAD \quad (1)$$

$$LAD \text{ (d)} = [(LAI_1 + LAI_2) \times (t_2 - t_1)]/2 \quad (2)$$

where TDM is total dry matter, LAI_1 and LAI_2 are the leaf area indexes (i.e., one-sided green leaf area per unit ground surface area) at times t_1 and t_2 , respectively, and t_1 and t_2 represent the times of the first and second harvest, respectively.

The specific leaf weight (SLW_a = leaf disc dry weight/fresh leaf disc area) and chlorophyll content (Chl_a) were measured at the anthesis stage from the ear leaf. To measure SLW_a , 20 leaf discs (6 mm in diameter) were taken from each sampled leaf with a leaf punch and oven dried until they reached a constant weight. Chl_a was measured with a SPAD-502 meter. Ovule number per ear (ON_{pe}) was counted from five randomly collected primary ears per subplot before anthesis.

The contribution of pre-anthesis dry matter to kernel (CPDMK) and current assimilate transfer to kernels (CATK) were estimated according to Min *et al.* (2011):

$$CPDMK \text{ (}\% \text{)} = (DMT/KYP) \times 100 \quad (3)$$

$$CATK \text{ (g plant}^{-1}\text{)} = KYP - DMT \quad (4)$$

where DMT (g plant⁻¹) is dry matter translocation (= dry matter at anthesis - dry matter at maturity of all vegetative parts) and KYP is kernel yield per plant at maturity. KYP was measured at maturity from the middle two rows (4-m length) within the 6-m² central area of each subplot, and it was computed assuming a 14% moisture content.

Root-to-shoot ratio (R/S_f) and biomass yield (BY_f) (including root, shoot, and ear) were measured per plant on a dry weight basis after the final harvest. Nitrogen use efficiency was calculated according to Bundy and Carter (1988).

$$NUE = \frac{\text{Kernel yield (kg)}}{\text{N used (kg)}} \quad (5)$$

Soil pH dynamics were assessed only in 2012. Following the method of CRCV (2006), soil pH was measured in the topsoil (0–30 cm depth) with a port-

able pH meter. Measurements were made daily for 1 week after both the first and second urea applications and thereafter at 10-day intervals.

Data were subjected to analysis of variance, which is appropriate for a split-plot design with factorial randomized complete block arrangements. Differences in mean values were analyzed using the Fisher's protected least-significance-difference procedure, with differences considered to be significant at $p < 0.05$. Path analyses were carried out on the traits considered as determinants of the yield, according to Dewey and Lu (1959). The CropStat ver. 7.2, SPSS ver. 16.0, and Agres-1 statistical packages were used for the analyses.

Results and Discussion

Soil pH

The initial soil pH was 6.7, but after the application of urea the pH changed (Fig. 1). Under the supra-optimal nitrogen application rate, the pH increased rapidly and the soil became alkaline within 3 days and remained so for few days; thereafter, the pH decreased gradually and after 4 weeks it was at the minimum value of 6.17, after both the first and second urea applications. With optimal nitrogen application, the pH initially increased slightly but soon returned to the initial value. The pH was more or less unchanged in the case of no urea application.

These findings are in accord with those of Tong and Xu (2012), who reported that addition of urea increased the soil pH during the early stages due to H^+ consumption during hydrolysis. This, in turn, caused an increase in the ammonia-oxidizing bacteria population, which accelerated nitrification and soil acidification.

Chlorophyll content

Water stress significantly reduced the leaf Chl_a content under both drought conditions (Table 1). Pioneer 30B80 produced significantly more Chl_a than Suwan 4452 in both years. The Chl_a value was drastically reduced under the zero nitrogen condition, whereas the plants under supra-optimal conditions performed similarly to those under optimal conditions in 2011 but better than in 2012. Pandey *et al.* (2000) also observed that leaf chlorophyll content increased as nitrogen level increased.

The Chl_a of the two varieties responded differently to water level in both years (Table 2), nitrogen level in

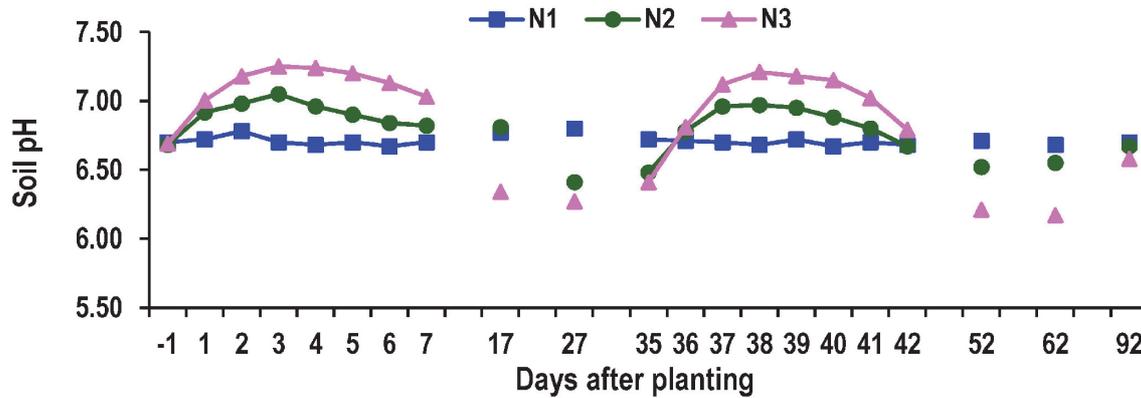


Fig. 1. Dynamics of soil pH under different levels of nitrogen applied as urea in 2012. The lines denote the initial 7-day periods after the first and second urea applications. N1, N2, and N3: 0, 160, and 320 kg N ha⁻¹, respectively.

Table 1. Main effects of water regime, variety, and nitrogen levels on agrophysiological traits of maize

Treatment	SLW _a (g m ⁻²)		Chl _a (SPAD score)		R/S _f (g g ⁻¹)		ON _{pe} (no. ear ⁻¹)		BY _f (g plant ⁻¹)	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
Water regime										
W1	47.47 ^b	48.24 ^b	54.18 ^a	50.05 ^a	0.13	0.12 ^b	673.00 ^a	660.58 ^a	325.17 ^a	295.52 ^a
W2	49.51 ^a	50.58 ^a	47.02 ^b	40.42 ^b	0.14	0.15 ^a	624.06 ^b	611.39 ^b	279.43 ^b	195.45 ^b
<i>F</i> test (W)	*	*	**	**	ns	**	*	*	**	**
Variety										
V1	50.26 ^a	50.31 ^a	51.44 ^a	46.09 ^a	0.17 ^a	0.17 ^a	653.99 ^a	641.49 ^a	320.90 ^a	264.94 ^a
V2	46.72 ^b	48.50 ^b	49.76 ^b	44.38 ^b	0.10 ^b	0.10 ^b	643.07 ^b	630.49 ^b	283.70 ^b	226.02 ^b
<i>F</i> test (V)	**	**	*	**	**	**	*	*	**	**
Nitrogen level										
N1	45.93 ^c	46.45 ^c	43.76 ^b	37.83 ^c	0.10 ^c	0.11 ^b	624.83 ^c	611.58 ^c	227.43 ^c	177.54 ^c
N2	50.99 ^a	51.38 ^a	53.50 ^a	48.09 ^b	0.17 ^a	0.17 ^a	673.75 ^a	661.88 ^a	359.76 ^a	286.26 ^a
N3	48.55 ^b	50.39 ^b	54.54 ^a	49.79 ^a	0.14 ^b	0.12 ^b	647.00 ^b	634.50 ^b	319.71 ^b	272.65 ^b
<i>F</i> test (N)	**	**	**	**	**	**	**	**	**	**

Note: The short pre-anthesis drought occurred in 2011 and the prolonged flowering-stage drought occurred in 2012; W1: well-watered; W2: water-stressed; V1: Pioneer 30B80; V2: Suwan 4452; N1, N2, and N3: 0, 160, and 320 kg N ha⁻¹, respectively; SLW_a: specific leaf weight at the anthesis stage; Chl_a: chlorophyll content at the anthesis stage; R/S_f: root-to-shoot ratio at the final harvest; ON_{pe}: ovule number per primary ear; BY_f: biomass yield at the final harvest. Means within a column with the same or no letters are not significantly different at $P < 0.05$ based on Fisher's protected least significance difference test. * $P < 0.05$; ** $P < 0.01$; ns, not significant.

both years (Table 3), and their interaction in 2012 (Table 4). Suwan 4452 was more susceptible to drought stress, and the reduction of Chl_a in this variety was more severe than in Pioneer 30B80 under single or simultaneous water and nitrogen stress. Higher Chl_a content in Pioneer 30B80 might be influenced by its

CO₂ assimilation and radiation use efficiency (Muchow and Sinclair, 1994).

Specific leaf weight

The SLW_a was significantly increased by water stress in both years (Table 1). This increase might be

Table 2. Effects of water regime \times variety interaction on agrophysiological traits of maize

Treatment	SLW _a (g m ⁻²)		Chl _a (SPAD score)		R/S _f (g g ⁻¹)		ON _{pe} (no. ear ⁻¹)		BY _f (g plant ⁻¹)	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
W1 \times V1	49.57 ^b	49.32 ^b	54.84 ^a	50.77 ^a	0.17	0.16 ^b	679.11 ^a	667.11 ^a	344.39 ^a	316.68 ^a
W1 \times V2	45.37 ^d	47.16 ^c	53.51 ^a	49.33 ^b	0.09	0.08 ^d	666.89 ^a	654.06 ^a	305.95 ^b	274.36 ^b
W2 \times V1	50.95 ^a	51.30 ^a	48.03 ^b	41.41 ^c	0.18	0.18 ^a	628.86 ^b	615.86 ^b	297.42 ^b	213.21 ^c
W2 \times V2	48.06 ^c	49.85 ^b	46.01 ^c	39.43 ^d	0.10	0.11 ^c	619.25 ^b	606.92 ^b	261.45 ^c	177.69 ^d
F test	*	*	*	*	ns	*	*	*	*	*

Note: The short pre-anthesis drought occurred in 2011 and the prolonged flowering-stage drought occurred in 2012; W1: well-watered; W2: water-stressed; V1: Pioneer 30B80; V2: Suwan 4452; SLW_a: specific leaf weight at the anthesis stage; Chl_a: chlorophyll content at the anthesis stage; R/S_f: root-to-shoot ratio at the final harvest; ON_{pe}: ovule number per primary ear; BY_f: biomass yield at the final harvest. Means within a column with the same or no letters are not significantly different at $P < 0.05$ based on Fisher's protected least significance difference test. * $P < 0.05$; ** $P < 0.01$; ns, not significant.

Table 3. Effects of variety \times nitrogen interaction on agrophysiological traits of maize

Treatment	SLW _a (g m ⁻²)		Chl _a (SPAD score)		R/S _f (g g ⁻¹)		ON _{pe} (no. ear ⁻¹)		BY _f (g plant ⁻¹)	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
V1 \times N1	47.80 ^c	47.66 ^d	45.17 ^b	39.75 ^d	0.13 ^c	0.13 ^c	631.34 ^{de}	616.84 ^{de}	252.48 ^d	209.70 ^d
V1 \times N2	51.97 ^a	52.18 ^a	54.10 ^a	48.65 ^{bc}	0.21 ^a	0.21 ^a	682.84 ^a	670.34 ^a	381.47 ^a	305.54 ^a
V1 \times N3	51.02 ^{ab}	51.10 ^{ab}	55.05 ^a	49.87 ^a	0.18 ^b	0.17 ^b	647.79 ^c	637.29 ^{bc}	328.76 ^b	279.59 ^b
V2 \times N1	44.06 ^e	45.25 ^e	42.35 ^c	35.90 ^e	0.07 ^e	0.10 ^d	618.33 ^e	606.33 ^e	202.39 ^e	145.39 ^e
V2 \times N2	50.01 ^b	50.58 ^{bc}	52.90 ^a	47.53 ^c	0.13 ^c	0.13 ^c	664.67 ^b	653.42 ^{ab}	338.05 ^b	266.98 ^c
V2 \times N3	46.09 ^d	49.69 ^c	54.03 ^a	49.72 ^{ab}	0.09 ^d	0.07 ^e	646.21 ^{cd}	631.71 ^{cd}	310.66 ^c	265.70 ^c
F test	**	*	*	**	**	**	*	*	*	**

Note: The short pre-anthesis drought occurred in 2011 and the prolonged flowering-stage drought occurred in 2012; V1: Pioneer 30B80; V2: Suwan 4452; N1, N2, and N3: 0, 160, and 320 kg N ha⁻¹, respectively; SLW_a: specific leaf weight at the anthesis stage; Chl_a: chlorophyll content at the anthesis stage; R/S_f: root-to-shoot ratio at the final harvest; ON_{pe}: ovule number per primary ear; BY_f: biomass yield at the final harvest. Means within a column with the same or no letters are not significantly different at $P < 0.05$ based on Fisher's protected least significance difference test. * $P < 0.05$; ** $P < 0.01$; ns, not significant.

due to the production of a thick waxy layer on the leaf surface to protect the plant from desiccation and minimize injury (Premachandra *et al.*, 1991) or due to the higher concentration of solute resulting from cell size reduction under water stress (Save *et al.*, 1993). Application of nitrogen significantly increased SLW_a in both varieties (Table 3). According to McAdam *et al.* (1989), nitrogen increased mesophyll cell number more than epidermal cell number, resulting in more cells adjacent to each abaxial epidermal cell, which

illustrates the interaction of mesophyll cell division and epidermal cell elongation. Although Peng *et al.* (1993) found that SLW was correlated with leaf nitrogen, in the present study supra-optimal nitrogen produced lower SLW_a, which may have led to the significantly lower NAR_v under the supra-optimal than optimal nitrogen conditions (Fig. 2). Pioneer 30B80 produced higher SLW_a, which may be related to this variety's higher NAR_v than that of Suwan 4452.

Table 4. Effects of water regime \times variety \times nitrogen interaction on agrophysiological traits of maize

Treatment	SLW _a (g m ⁻²)		Chl _a (SPAD score)		R/S _f (g g ⁻¹)		ON _{pe} (no. ear ⁻¹)		BY _f (g plant ⁻¹)	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
W1 \times V1 \times N1	46.53	45.49	48.77	44.80 ^c	0.13	0.12 ^{de}	648.00 ^{de}	634.00 ^{ef}	257.33 ^e	250.04 ^d
W1 \times V1 \times N2	51.22	51.45	57.27	53.07 ^{ab}	0.20	0.19 ^b	714.00 ^a	702.00 ^a	418.67 ^a	363.56 ^a
W1 \times V1 \times N3	50.96	51.01	58.50	54.43 ^a	0.18	0.15 ^c	675.33 ^c	665.33 ^{bc}	357.17 ^{bc}	336.44 ^b
W1 \times V2 \times N1	42.05	43.08	46.20	40.80 ^d	0.06	0.08 ^f	625.33 ^f	613.33 ^{fg}	221.50 ^f	182.79 ^g
W1 \times V2 \times N2	49.54	50.03	56.37	52.60 ^b	0.12	0.12 ^{de}	698.67 ^{ab}	687.17 ^{ab}	376.77 ^b	331.24 ^b
W1 \times V2 \times N3	44.53	48.36	57.97	54.60 ^a	0.09	0.06 ^g	676.67 ^{bc}	661.67 ^{cd}	319.58 ^d	309.04 ^c
W2 \times V1 \times N1	49.06	49.83	41.57	34.70 ^e	0.13	0.13 ^d	614.67 ^f	599.67 ^g	247.63 ^e	169.36 ^g
W2 \times V1 \times N2	52.72	52.90	50.93	44.23 ^c	0.22	0.23 ^a	651.67 ^d	638.67 ^{de}	344.27 ^c	247.52 ^d
W2 \times V1 \times N3	51.07	51.18	51.60	45.30 ^c	0.19	0.18 ^b	620.25 ^f	609.25 ^g	300.35 ^d	222.74 ^e
W2 \times V2 \times N1	46.06	47.41	38.50	31.00 ^f	0.07	0.11 ^e	611.33 ^f	599.33 ^g	183.27 ^g	107.98 ^h
W2 \times V2 \times N2	50.48	51.12	49.43	42.47 ^d	0.14	0.15 ^c	630.67 ^{ef}	619.67 ^{efg}	299.33 ^d	202.72 ^f
W2 \times V2 \times N3	47.65	51.02	50.10	44.83 ^c	0.10	0.08 ^f	615.75 ^f	601.75 ^g	301.74 ^d	222.36 ^e
F test	ns	ns	ns	*	ns	*	*	*	*	*

Note: The short pre-anthesis drought occurred in 2011 and the prolonged flowering-stage drought occurred in 2012; W1: well-watered; W2: water-stressed; V1: Pioneer 30B80; V2: Suwan 4452; N1, N2, and N3: 0, 160, and 320 kg N ha⁻¹, respectively; SLW_a: specific leaf weight at the anthesis stage; Chl_a: chlorophyll content at the anthesis stage; R/S_f: root-to-shoot ratio at the final harvest; ON_{pe}: ovule number per primary ear; BY_f: biomass yield at the final harvest. Means within a column with the same or no letters are not significantly different at $P < 0.05$ based on Fisher's protected least significance difference test. * $P < 0.05$; ** $P < 0.01$; ns, not significant.

Net assimilation rate

The NAR_v was higher in Pioneer 30B80 than Suwan 4452 in both years (Fig. 2a, b). Well-watered and optimal nitrogen conditions had a positive influence on NAR_v, whereas supra-optimal nitrogen, zero nitrogen, and water-stressed conditions significantly decreased NAR_v, particularly during the prolonged flowering-stage drought, similar to the findings of Owen and Watson (1956). Optimal nitrogen resulted in greater NAR_v in Pioneer 30B80 under all conditions, except under the well-watered condition in 2012, when the two varieties had similar values (Fig. 2c, d).

Although the leaf area was somewhat smaller in Pioneer 30B80 at the early stage due to slower leaf expansion (about 10%), this variety had more NAR_v than Suwan 4452, which may have resulted from its greater Chl_a and SLW_a (Table 1). However, greater Chl_a under the supra-optimal nitrogen condition (Table 1) could not increase NAR_v as much as under optimal nitrogen. This finding might be due to less photosynthesis under the supra-optimal nitrogen condition resulting from lower stomatal conductance through

abscisic acid signaling or an imbalance of K⁺ and Cl⁻ or less availability of other related ions due to fluctuating soil pH (Fig. 1).

Ovule number

Water and nitrogen stress significantly reduced the ON_{pe}, and Pioneer 30B80 contained relatively higher ON_{pe} under all conditions in both years (Tables 1–4). Optimal nitrogen and well-watered conditions enhanced ovule setting, suggesting that nitrogen might accelerate cell differentiation. A greater reduction of ON_{pe} was observed under prolonged flowering-stage drought, and both varieties responded similarly to water levels (Table 2). As reported by Uhart and Andrade (1995), nitrogen levels significantly affected the ON_{pe}, and ON_{pe} of each variety responded differently among the three nitrogen conditions (Table 3). The ON_{pe} was highest in Pioneer 30B80 with optimal nitrogen, which might be due to a higher crop growth rate resulting from higher NAR_v, as also noted by Uhart and Andrade (1995).

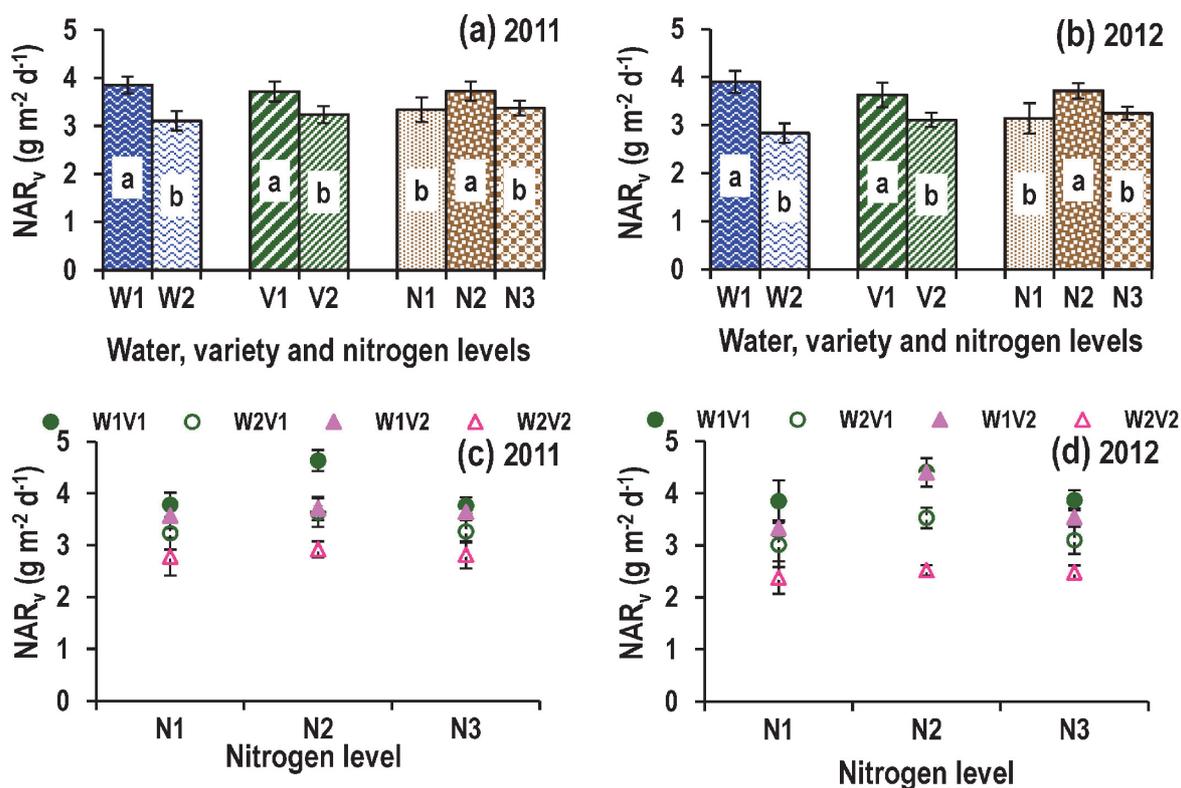


Fig. 2. Net assimilation rate at the vegetative stage (NAR_v) as affected by the main effects of water (W1: well-watered, W2: water-stressed), variety (V1: Pioneer 30B80, V2: Suwan 4452), and nitrogen (N1, N2, and N3: 0, 160, and 320 kg N ha⁻¹, respectively) in 2011 (a) and 2012 (b) and by their interaction in 2011 (c) and 2012 (d). Within the same treatment, bars with different letters were significantly different ($P < 0.05$) according to Fisher's protected least significance difference test.

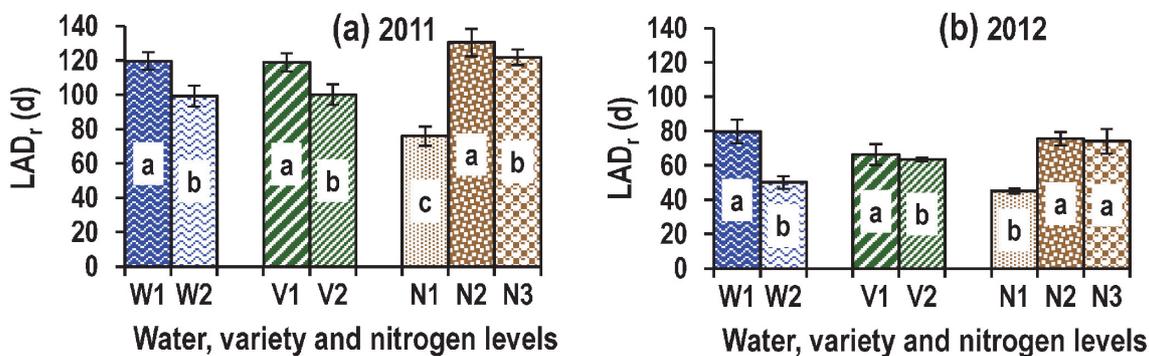


Fig. 3. Leaf area duration at reproductive stage (LAD_r) as affected by the main effects of water (W1: well-watered, W2: water-stressed), variety (V1: Pioneer 30B80, V2: Suwan 4452), and nitrogen (N1, N2, and N3: 0, 160 and 320 kg N ha⁻¹, respectively) in 2011 (a) and 2012 (b). Within the same treatment, bars with different letters were significantly different ($P < 0.05$) according to Fisher's protected least significance difference test.

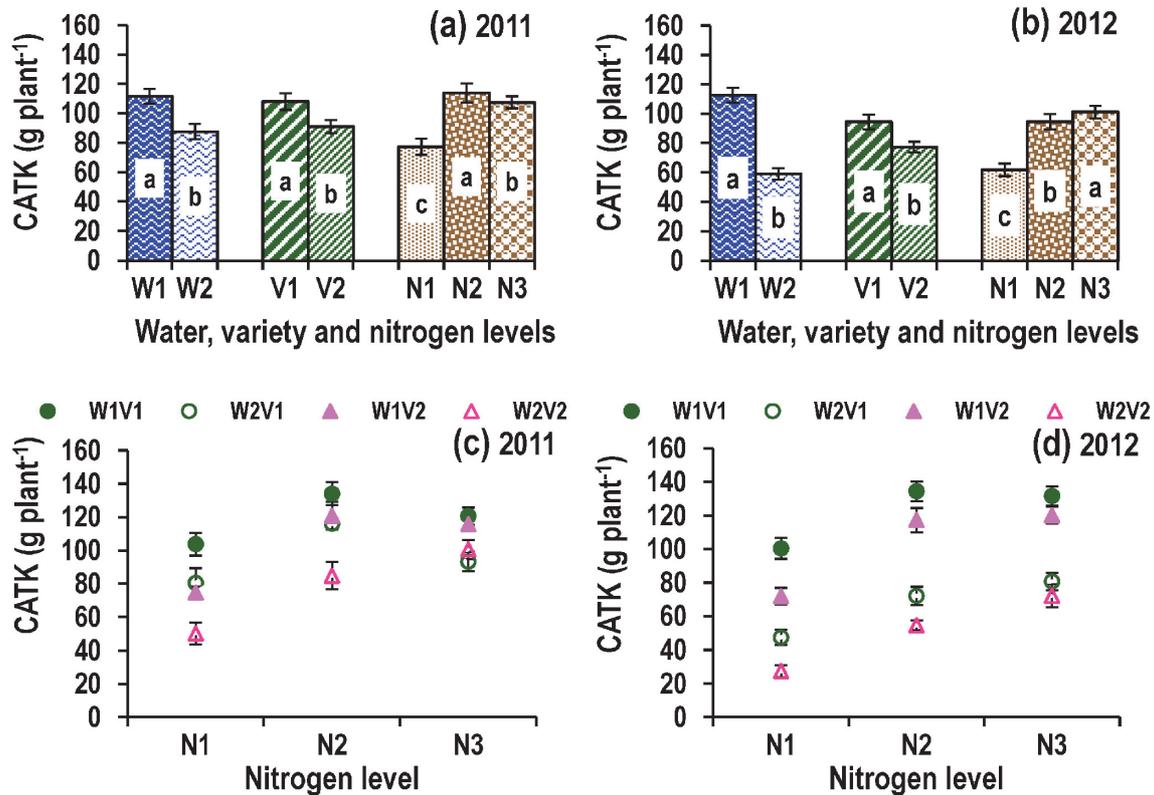


Fig. 4. Current assimilate transfer to kernels (CATK) as affected by the main effects of water (W1: well-watered, W2: water-stressed), variety (V1: Pioneer 30B80, V2: Suwan 4452), and nitrogen (N1, N2, and N3: 0, 160, and 320 kg N ha⁻¹, respectively) in 2011 (a) and 2012 (b) and by their interaction effect in 2011 (c) and 2012 (d). Within the same treatment, bars with different letters were significantly different ($P < 0.05$) according to Fisher's protected least significance difference test.

Leaf area duration

The LAD_r was significantly affected by water, variety, and nitrogen (Fig. 3). Comparatively higher water and nutrient (especially nitrogen) uptake through greater root mass might have assisted plants in maintaining green leaves for a longer period. The LAD_r also depends on initial LAI and leaf relative senescence rate. As a result, the LAD_r was higher in well-watered than in water-stressed plants in both years, and it was decreased more under prolonged flowering-stage drought (Fig. 3a, b). This latter result may have occurred for several reasons: (1) more leaf senescence in relation to the higher rate of dry matter translocation to contribute to kernel filling (see Fig. 5a, b); (2) the deprivation of amino acids and more nitrogen translocation; or (3) greater respiration losses under prolonged drought. Although the initial LAI was lower in Pioneer 30B80 (about 15 and 10% less in

2011 and 2012, respectively), this variety was able to maintain a longer LAD_r than that of Suwan 4452 due to its lower relative senescence rate. Greater leaf longevity might supply more assimilate during kernel filling, which may increase stress tolerance, as reported by Tollenaar and Wu (1999). Nitrogen fertilization inhibits the relative senescence rate and extends LAD_r . Nevertheless, the LAD_r was lower under supra-optimal than optimal nitrogen conditions, and it was actually due to lower initial LAI.

Current assimilate transfer to kernels

Current assimilate production and transfer during the kernel-filling period mostly depends on the availability of green leaves in a plant. Accordingly, we found that water and nitrogen stress significantly reduced CATK in both years (Fig. 4a, b). The greater LAD_r of Pioneer 30B80 allowed it to achieve greater

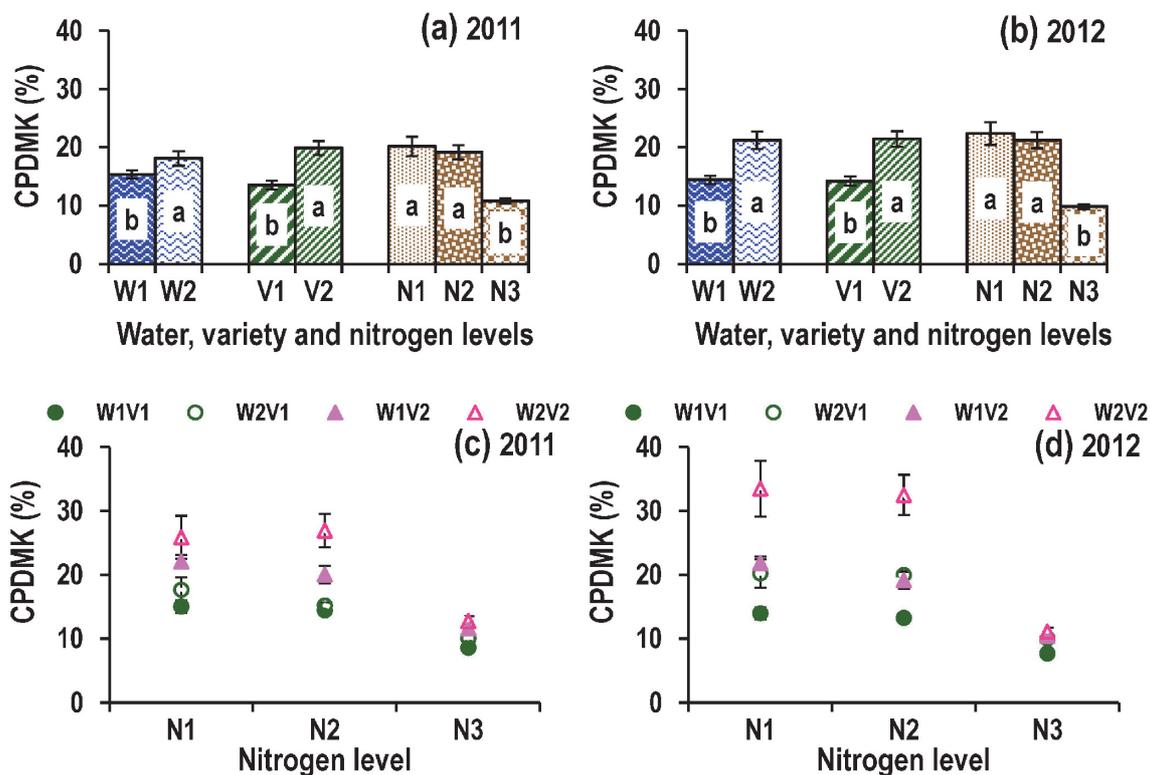


Fig. 5. Contribution of pre-anthesis dry matter to kernel (CPDMK) as affected by the main effects of water (W1: well-watered, W2: water-stressed), variety (V1: Pioneer 30B80, V2: Suwan 4452), and nitrogen (N1, N2, and N3: 0, 160, and 320 kg N ha⁻¹, respectively) in 2011 (a) and 2012 (b) and by their interaction effect in 2011 (c) and 2012 (d). Within the same treatment, bars with different letters were significantly different ($P < 0.05$) according to Fisher's protected least significance difference test.

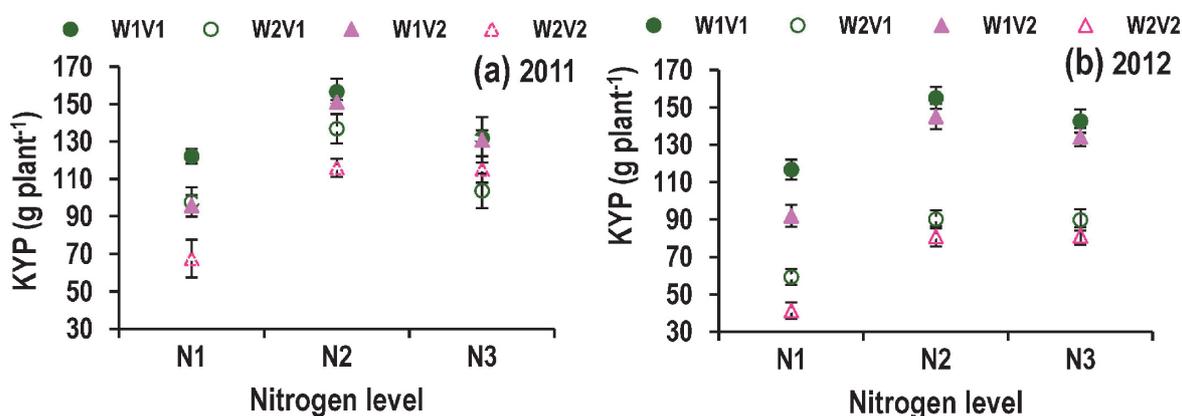


Fig. 6. Kernel yield per plant (KYP) as affected by interaction effect of water (W1: well-watered, W2: water-stressed), variety (V1: Pioneer 30B80, V2: Suwan 4452), and nitrogen (N1, N2, and N3: 0, 160, and 320 kg N ha⁻¹, respectively) in 2011 (a) and 2012 (b).

CATK than Suwan 4452, which partially corroborates the findings of Worku *et al.* (2012). The CATK also depends on the sink size. As expected, we found that CATK was lower under supra-optimal than optimal nitrogen conditions in 2011, whereas the conflicting results obtained in 2012 might reflect the impact of prolonged water stress (Fig. 4d). Suwan 4452 showed less efficient CATK under both water and nitrogen stress, whereas Pioneer 30B80 was less efficient under the supra-optimal nitrogen condition (Fig. 4c, d).

Contribution of pre-anthesis dry matter to kernel

When green leaves fail to support the sink (kernel) demand for assimilate, the plant needs to translocate available pre-anthesis reserve dry matter from the vegetative parts to kernels. As a result, CPDMK was higher under water and nitrogen stress conditions and in Suwan 4452 in both years (Fig. 5a, b), reflecting this variety's shorter LAD_r (Fig. 3a, b). Despite the greater LAD_r under the optimal nitrogen condition (Fig. 3a, b), the CPDMK values were similar between the zero and optimal nitrogen conditions (Fig. 5a, b), particularly under the water-stress condition (Fig. 5c, d). These results might be due to the much higher demand of the sink during kernel filling under the optimal nitrogen condition, which was partially created by greater ovule setting (Table 1). The CPDMK was higher in Suwan 4452 than in Pioneer 30B80 across all water and nitrogen levels (Fig. 5c, d), although the KYP of Suwan 4452 was lower (see Fig. 6a, b). Maize varieties with greater CPDMK tend to have lower yields (Min *et al.*, 2011).

Root-to-shoot ratio

More root dry matter is a desirable characteristic for tolerance against water and nitrogen stress (Pandey *et al.*, 2000). Pioneer 30B80 maintained a greater R/S_f than Suwan 4452 in both years (Table 1), under water stress alone or under simultaneous water and nitrogen stresses (Tables 2–4). Water stress reduced shoot dry matter more than root dry matter, which caused higher R/S_f under the water-stressed condition as compared to the well-watered condition (Table 1). The prolonged drought in 2012 had a greater detrimental effect on shoot dry matter than did the short drought in 2011, such that R/S_f under water stress in 2012 was greater than that in 2011. In contrast, zero and supra-optimal nitrogen had more detrimental effects on roots than shoots, which caused lower R/S_f in these treatments

than under the optimal nitrogen condition. Low photo-assimilate production and translocation to roots under zero nitrogen may have constrained proper root growth. Optimal nitrogen is conducive to greater root development (Pandey *et al.*, 2000), although excessive nitrogen application can inhibit root growth in maize (Wang *et al.*, 2008).

Kernel yield per plant

The KYP was decreased due to water and nitrogen stresses and under the supra-optimal nitrogen condition in both 2011 and 2012 (Fig. 6a, b). As compared to Pioneer 30B80, KYP was severely decreased in Suwan 4452 under both water and nitrogen stresses, which may have resulted from less ovule (Table 1) and kernel setting (about 5 and 6% less in 2011 and 2012, respectively) in this variety. Hokmalipour and Darbandi (2011) reported a similar trend under nitrogen stress, whereas Su *et al.* (2010) suggested that kernel yield was limited more by sink size. Pioneer 30B80 produced the highest KYP under optimal nitrogen and well-watered conditions, whereas Suwan 4452 had the lowest KYP under zero nitrogen and water-stressed conditions, with the yield severely reduced (72%, compared to control) due to prolonged drought in 2012. Pioneer 30B80 produced higher KYP than Suwan 4452 under all water levels, which may have resulted from a greater number of ovules and higher NAR assisted by higher Chl_a, SLW_a, LAD, CATK, and root volume in this variety.

Biomass yield

The highest BY_f was obtained from Pioneer 30B80 under well-watered and optimal nitrogen conditions (Table 1), which was attributed to both higher root and shoot dry matter as a result of higher NAR_v (Fig. 2). The BY_f was lower in 2012 than in 2011 due to the detrimental effects of prolonged drought. Owen and Watson (1956) also observed that the leaf area, total dry matter production, and NAR of sugar beet were all reduced by a period of prolonged water stress. Higher root dry matter made the greatest contribution to the higher BY_f in Pioneer 30B80 (about 6% higher in both years), and Suwan 4452 produced the lowest BY_f under simultaneous stresses (Table 4). Under water-stress conditions, soil nutrients become less available (Pandey *et al.*, 2000), and plant total dry matter may be reduced due to less root proliferation. The BY_f was severely reduced due to water stress under zero ni-

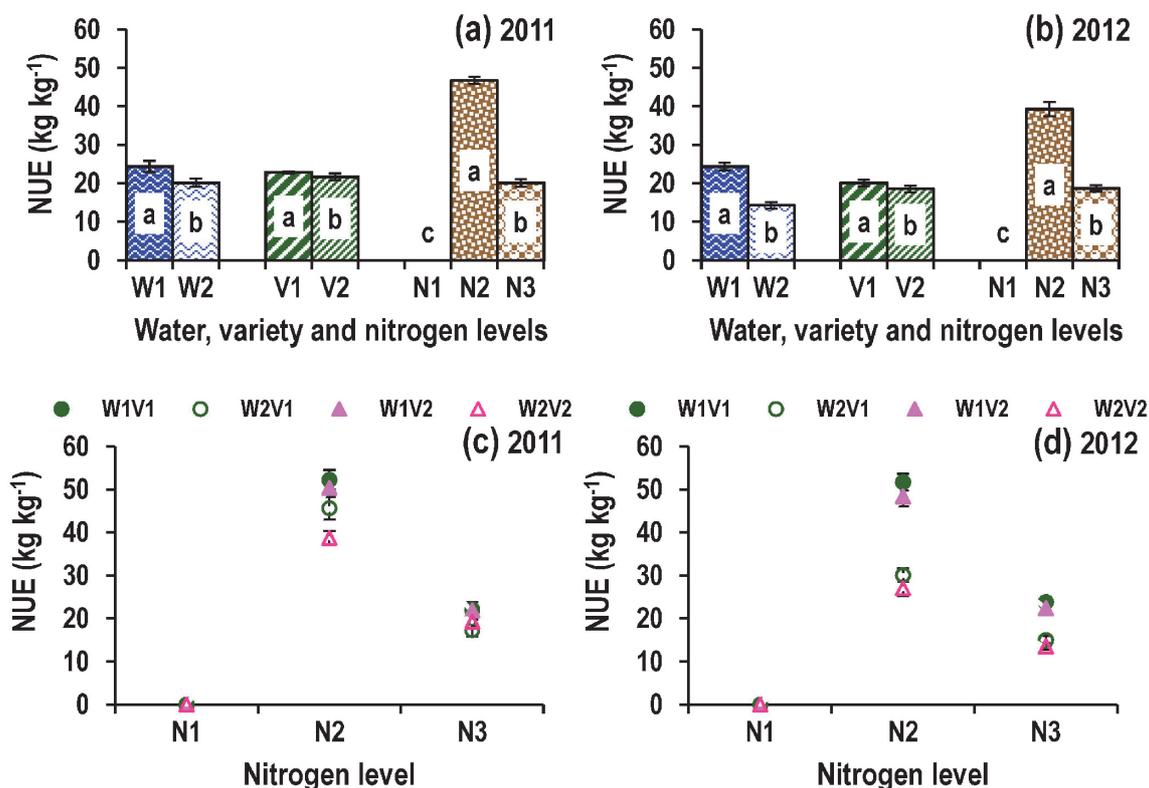


Fig. 7. Nitrogen use efficiency (NUE) as affected by the main effects of water (W1: well-watered, W2: water-stressed), variety (V1: Pioneer 30B80, V2: Suwan 4452), and nitrogen (N1, N2, and N3: 0, 160, and 320 kg N ha⁻¹, respectively) in 2011 (a) and 2012 (b) and by their interaction effect in 2011 (c) and 2012 (d). Within the same treatment, bars with different letters were significantly different ($P < 0.05$) according to Fisher's protected least significance difference test.

trogen as compared to optimal or supra-optimal nitrogen conditions. Pandey *et al.* (2000) also noted that nitrogen has ameliorative effects against drought in maize.

Nitrogen use efficiency

Sufficient water and nitrogen increased the nitrogen use efficiency in maize. Water stress decreased nitrogen use efficiency, and this effect was more pronounced under prolonged drought (Fig. 7a, b), perhaps due to the limited availability of nitrogen for a long period as a result of excess volatile losses of nitrogen from the soil. Optimal nitrogen increased nitrogen use efficiency in both years, whereas supra-optimal nitrogen decreased it. Pioneer 30B80 performed better than Suwan 4452 under all conditions, showing higher nitrogen use efficiency under optimal nitrogen and water conditions. The worst nitrogen use efficiency was recorded in Suwan 4452 under simultaneous water

and nitrogen stresses.

Muchow (1998) also reported that nitrogen use efficiency declined when nitrogen was supplied at supra-optimal levels or when growth was limited by moisture stress. Bennett *et al.* (1986) suggested that high nitrogen caused greater sensitivity to drought in some plants. The reduction of nitrogen use efficiency under supra-optimal nitrogen caused lower KYP, and it might have been due to an excess of NH_4^+ -N penetrating roots during the first few days after each urea application, which can trigger higher pH (Hoffmann and Kosegarten, 1995) and lead to abscisic acid signaling (Morgan, 1986). Excess NH_4^+ can also be substituted for K^+ , accompanied by an increase in Cl^- (Britto and Kronzucker, 2002), which causes stomatal closure and consequently less photosynthesis.

Correlation and path coefficient analysis

The correlations between KYP and LAD_r , Chl_a ,

Table 5. Indirect effects via various paths of six traits on kernel yield per plant and their correlation in 2011 and 2012

Trait	Indirect effect via												Total correlation with KYP	
	LAD _r		Chl _a		NAR _v		SLW _a		ON _{pe}		CATK _r		2011	2012
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012		
LAD _r			-0.20	0.36	0.04	0.10	0.02	-0.03	0.10	0.24	0.50	0.33	0.77	0.95
Chl _a	0.25	-0.05			0.09	0.09	0.03	-0.03	0.12	0.22	0.64	0.33	0.87	0.94
NAR _v	0.10	-0.04	-0.16	0.27			0.02	-0.00	0.12	0.24	0.53	0.30	0.74	0.89
SLW _a	0.06	-0.02	-0.08	0.11	0.03	0.00			0.04	0.07	0.33	0.05	0.47	0.13
ON _{pe}	0.20	-0.05	-0.21	0.31	0.12	0.11	0.03	-0.02			0.60	0.31	0.88	0.93
CATK _r	0.22	-0.05	-0.23	0.35	0.10	0.10	0.05	-0.01	0.12	0.23			0.97	0.99

Note: The short pre-anthesis drought occurred in 2011 and the prolonged flowering-stage drought occurred in 2012; LAD_r: leaf area duration at the reproductive stage; Chl_a: chlorophyll content at the anthesis stage; NAR_v: net assimilation rate at the vegetative stage; SLW_a: specific leaf weight at the anthesis stage; ON_{pe}: ovule number per primary ear; CATK: current assimilate transfer to kernels; KYP: kernel yield per plant.

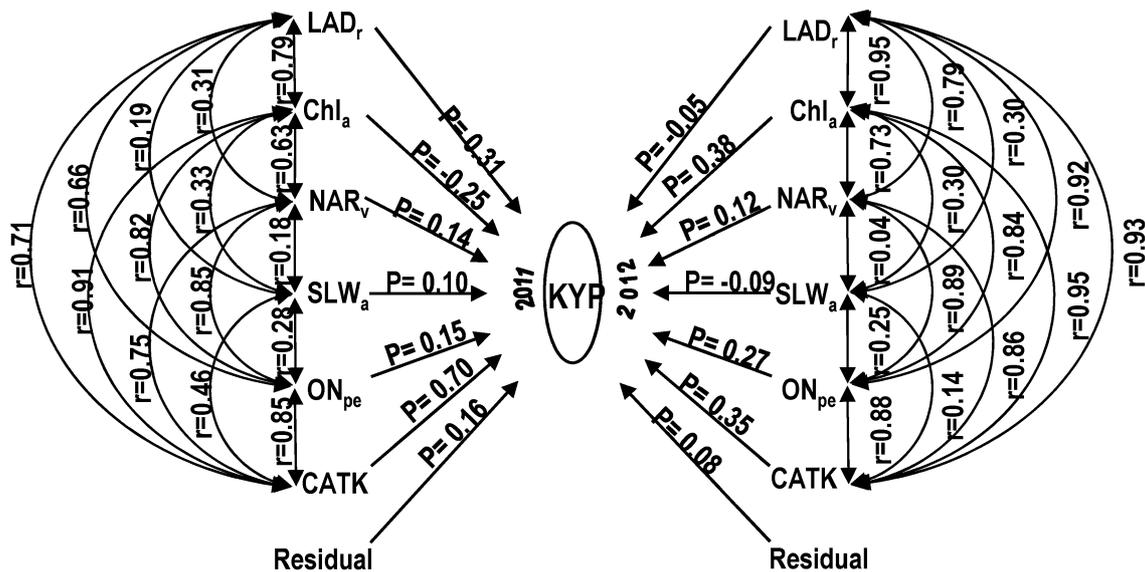


Fig. 8. Path diagram and association of different characteristics during experiments in 2011 and 2012. Single arrows denote the direct effect (P) on kernel yield per plant (KYP); double arrows denote the correlation coefficient (r) between traits. LAD_r: leaf area duration at the reproductive stage; Chl_a: chlorophyll content at the anthesis stage; NAR_v: net assimilation rate at the vegetative stage; SLW_a: specific leaf weight at the anthesis stage; ON_{pe}: ovule number per primary ear; CATK: current assimilate transfer to kernels.

NAR_v, ON_{pe}, and CATK (but not SLW_a) were positive and highly significant in both years, especially under prolonged flowering-stage drought (Table 5). The interrelationships among those significant traits were also strong in both years (Fig. 8). The poor relation-

ship between SLW and grain yield under field conditions implies that caution must be exercised when using SLW as an index of photosynthetic capacity: SLW can be used as an indicator of photosynthetic capacity only when leaves share a similar life history.

Path coefficient analysis (Fig. 8) showed that the direct effect of CATK on KYP was very strong. KYP was directly affected by NAR_v and ON_{pe} in both years, whereas LAD_r and Chl_a had a direct effect on KYP in 2011 and 2012, respectively. SLW_a had little or no direct effect on KYP. LAD_r , Chl_a , and NAR_v had positive indirect effects on KYP via both ON_{pe} and CATK in both years (Table 5). SLW_a (in 2011) and ON_{pe} (in both years) also had positive indirect effects on KYP via CATK, and CATK had an indirect effect on KYP via Chl_a and NAR_v in both years.

Conclusions

Drought and nitrogen stresses, as well as supra-optimal nitrogen, had detrimental effects on plant growth and yield, especially on the roots and activity of leaves. Optimal nitrogen (160 kg N ha^{-1}) had positive effects and could ameliorate the adverse effects of drought through several mechanisms. Optimal nitrogen accelerated root growth to take up more water and nutrients and enhanced chlorophyll content, specific leaf weight, and leaf area duration, which in turn helped to increase the net assimilation rate and subsequently increased ovule setting, ultimately leading to increased kernel and biomass yield. Some varietal characters also lessened the detrimental effects of drought. A higher net assimilation rate with longer leaf area duration during the kernel-filling period and more root dry matter jointly helped the plants to produce and transfer more current assimilate to the kernels, which allowed Pioneer 30B80 to produce higher yield and made it less susceptible to both water and nitrogen stresses. Thus, using varieties that stay green longer and proper nitrogen management can help to maximize photoassimilate production in maize plants. This, in turn, will contribute to global food security and help to decrease atmospheric CO_2 and mitigate climate change by capturing and storing more carbon.

Acknowledgements

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