Kasetsart J. (Nat. Sci.) 48 : 676 - 688 (2014)

Growth and Physiological Responses to Supra-Optimal Nitrogen and Pre-Anthesis Drought Stress in Maize

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ABSTRACT

Water and nitrogen (N) are major limiting factors in the production of maize (*Zea mays* L.) in the tropics and subtropics. A field experiment was conducted during the dry season in 2010–11 at the National Corn and Sorghum Research Center, Thailand to determine the sole and interactive effects of N, water and variety on the growth, physiology and grain yield of maize. Two water regimes (well-watered and water-stressed) formed the main plots and two hybrids (Pioneer 30B80 and Suwan 4452) and three N levels (0 kg.ha^{-1}, 160 kg.ha^{-1} (optimal) and 320 kg.ha^{-1} (supra-optimal) were subplots with three replications using a split plot design and a factorial randomized complete block arrangement. Zero and supra-optimal N, and water-stress significantly reduced the green leaf area (37.86, 6.76 and 17.64%, respectively, at silking), root dry matter (RDM) (62.48, 15.66 and 48.83%, respectively), grain number (20.43, 11.96 and 16.76%, respectively) and grain yield (GY) (31.64, 14.07 and 19.20%, respectively) but not leaf rolling when compared to the control. The leaf relative water content (RWC) decreased (12.87%) significantly only with water-stress. The two hybrids responded differently to N with regard to leaf area—Suwan 4452 was more sensitive to zero N and Pioneer 30B80 was more sensitive to supra-optimal N. Suwan 4452 was lower in RDM (53.92%), grain number (12.90%), and GY (9.66%) than Pioneer 30B80, with the latter performing better under single or combined stress. Higher RDM levels in the surface soil during vegetative growth, the amount of kept green leaves and more grain and a higher RWC were exhibited as drought and N stress tolerance indicators.

Keywords: drought, nitrogen levels, maize varieties, physiology, growth and yield

INTRODUCTION

Drought caused by the erratic distribution of rainfall is one of the major causes of reduction in the world's maize yield (Bruce *et al*., 2002). Under rainfed agriculture, a satisfactory grain yield from a crop is dependent upon the variety and its ability to tolerate water stress (Banziger *et al*., 2000). Therefore, the environment can play an important role in the production by crop varieties (Derby *et al*., 2004). Horizontal improvement for crop production is becoming limited due to increasing population levels. Therefore, effort has to made for a vertical increase. Furthermore, the cost of production has to be reduced while inputs are optimized through proper management. Moreover, environmental pollution would be controlled (Derby *et al*., 2005) by minimizing the inappropriate application of nitrogen, which is highly volatile.

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Received date : 02/10/13 Accepted date : 24/05/14

Nitrogen can play an important role in determining plant responses to drought stress. Nitrogen deficiency constitutes one of the major yield-limiting factors for cereal production (Shah *et al.,* 2003) and the yield of maize was reduced 30% due to N deficiency during the vegetative stage (Subedi and Ma, 2005). In contrast, high nitrogen caused higher sensitivity toward drought in some plants (Bennett *et al.*, 1986; Morgan, 1986); though there can be less sensitivity (Ogren, 1985) or no sensitivity (Yambao and O'Toole, 1984).

Many physiological processes associated with maize growth may be enhanced by N supply and research has shown the importance of water and N interactions in optimizing maize productivity (Eck, 1984). Leaf area plays an important role in plant growth analysis (Mokhtarpour *et al.*, 2010) and in the plant's efficiency to produce biomass is dependent on leaf N (Muchow, 1988). Variation in the N supply by any means affects the crop growth, development and potential kernel set and grain yield (Pandey *et al.*, 2000a). N-deficient maize leaves are very sensitive to water deficits (Bennett *et al.*, 1986). It has been reported that nitrogen affects the carbohydrate metabolism, osmotic regulation, cell wall elasticity and synthesis of drought-induced signal substances in roots (Ogren, 1985; Morgan, 1986). Maize root is very sensitive to N and water stress, with N being important for optimizing plant growth by releasing substances such as organic acids (Renato and Paulo, 1997). Under drought conditions, photosynthesis may reduce noticeably due to a decrease in radiation interception associated with reduced leaf expansion and leaf rolling (Obeng-Bio *et al.*, 2011), foliar senescence (Mansouri-Far *et al.*, 2010) and to a reduction in C fixation per unit leaf area because of stomatal closure or a decline in carboxylation capacity (Bruce *et al.*, 2002).

Water and N stress under field conditions are common and understanding the interaction of these two inputs is important for efficient production of maize. However, there is very little evidence of an influence of nitrogen levels on maize response to drought stress. Therefore, it is worthwhile to study the N and pre-anthesis drought stress responses on maize growth, physiology, and grain yield.

MATERIALS AND METHODS

The field experiment was conducted during the regular dry period from December, 2010 to April, 2011 at the National Corn and Sorghum Research Center, Nakhon Ratchasima province, Thailand (14º38′ N, 101º19′ E, 387.92 m above mean sea level).

General experimental conditions

Soil

The top soil layer (0–30 cm) contained 75% clay, 10% silt, and 15% sand. The organic matter content was 2.28%, the pH was 6.8 and the P, K, Ca and Mg cotnetns were 72, 140, 2,400 and 210 $mg.kg^{-1}$, respectively. The second soil layer (30–60 cm) contained 83% clay, 6% silt and 11% sand and the organic matter content was 1.95%, the pH was 6.6 and the P, K, Ca and Mg contents were 7, 40, 1,920 and 170 $mg.kg^{-1}$, respectively.

Weather

Data were collected from the automatic weather station at the National Corn and Sorghum Research Center, Pak Chong located within 200 m of the experimental plot. Rainfall ranged from 0.5 to 32.3 mm, but mostly occurred during the late grain filling period and there was no noticeable rainfall during the water-stressed period (Figure 1). Relative humidity (RH) ranged from 44 to 88%.

Experimental details

Design

The experiment was laid out in a split plot design with factorial arrangements of the treatments with three replications. The individual plot size was 7.5 × 7.5 m. Two water regimes (W1 = Well-watered (control) and W2 = Water-stressed) were assigned to the main plots and the combination of two varieties (V1 = Pioneer 30B80 and V2 = Suwan 4452) and three N levels—N1 = 0 kg.ha^{-1}, N2= 160 kg.ha^{-1} (optimal & control) and N3 = 320 kg.ha^{-1} (supra-optimal)—were randomly assigned within each main plot. In each subplot, 10 rows of maize were planted.

Cultivation practices

Two weeks before starting the experiment, initial soil samples (0–30 and 30–60 cm depth) from the experimental site were taken to determine the physical and chemical properties. The land was prepared using a disc harrow, with ridges and furrows spaced at 75 cm. On 3 December 2010, two seeds per hill were planted in the ridge maintaining the spacing of 75 × 25 cm. Thinning was carried out 2 wk after planting (WAP), maintaining one plant per hill. Half of each nitrogen treatment was applied as urea at the base (N = 46%) and the other half was applied as a side dressing at 34 days after planting (DAP) at about the 8-leaf stage followed by irrigation (Feil *et al.*, 2005). To avoid the lateral flow of irrigation water, the water-stressed (WS) block was set up 10 m away from the well-watered (WW) block, with a deep drain surrounding the WS block, and a 5 m protection area surrounded the whole block. Promptly after planting, the entire experimental field was irrigated with approximately 40 mm of water for 3 hr using a sprinkler system to ensure the initial growth and establishment of the seedlings (Wongpila, 2008). Five more sprinkler irrigation sessions were provided at weekly intervals. Thereafter, furrow irrigation was provided at 7 d intervals to maintain the field capacity. In the WS treatment, irrigation was suspended from 35 to 64 DAP and then again irrigated was re-introduced up to physiological maturity as with the WW block. No noticeable disease or insect problems were detected during the experimental period and two sessions of weeding were applied manually.

Measurements

Soil moisture status

The soil moisture content (SMC) was monitored at 7 d intervals during the 30 d dry period using the gravimetric method (Ryan *et al.*, 2001). The moisture content was measured from the 0–30 and 30–60 cm soil layers and the SMC was computed on a dry weight basis.

Plant status

The nondestructive *in vivo* fully expanded green leaf area (FEGLA) was measured every week from five pre-selected plants. The total leaf area (green area only) was computed as the sum of (length × maximum width × 0.75) for each fully expanded green leaf (less than 50% of leaf surface yellow or dead) according to Muchow (1988). In the afternoon just before the end of the water stress period, leaf rolling (LR) was assessed from the

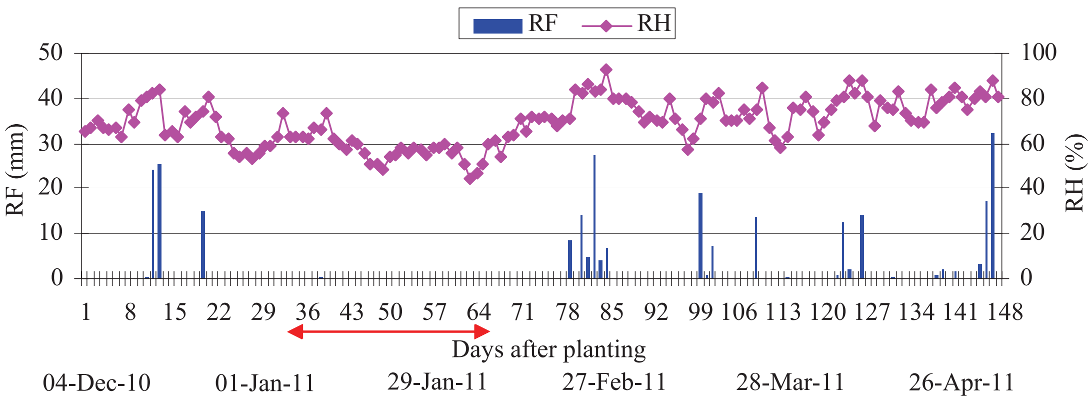

Figure 1 Rainfall (RF) and relative humidity (RH) for the period December 2010 to April 2011. (Double-headed arrow shows the water withholding period).

central two rows avoiding any plot border effect. LR was scored according to Banziger *et al.* (2000) on a scale from 1 to 5 (1 = unrolled, 2 = leaf rim starting to roll, 3 = leaf has a v-shape, 4 = rolled leaf rim covers part of the leaf blade and 5 = leaf is rolled into the shape of an onion leaf). The leaf relative water content (RWC) was determined according to the formula (Henson *et al*., 1989): RWC = (fresh weight – dry weight) / (turgid weight – dry weight) × 100. The RWC was measured just before the end of the water-stressed period on the youngest fully expanded leaf. Root dry matter (RDM) was measured from 0 to 40 cm soil depth during the anthesis period. Grain number (GNM) was defined as the count of the number of grains per square meter and calculated from five pre selected plants after final harvest. Grain yield (GY) was obtained from the two midrows in the central 4 m area on 6 m^2 within each plot by hand-harvesting. The GY was computed assuming 14% moisture content.

Statistical analysis

Growth and yield data were subjected to analysis of variance for a split plot design with a factorial randomized complete block design and the means were compared with Fisher's protected least significance difference (LSD) procedure at $P = 0.05$ (Steel and Torrie, 1980).

RESULTS

Soil moisture content

The SMC was always higher in the subsurface soil (30–60 cm) than the surface soil (0–30 cm) being 32.37 and 32.99%, respectively, at zero weeks after water withholding (WAWW) as shown in Figure 2. Then, the SMC reduced more sharply in the 0–30 cm layer than in the 30-60 cm layer during the first WAWW after which time the trend became similar. After two WAWW, the SMC reduced gradually and at four WAWW, it was more pronounced in the 0–30 cm layer which might have been due to the sudden high temperature when the crop was just in the pre-anthesis period.

Fully expanded green leaf area

Maize FEGLA was significantly reduced from three weeks after water withholding (at 8 WAP) to 16 WAP due to water stress across all the varieties and N levels (Figure 3). The reduction of the FEGLA was more pronounced at the end of the water stress period (23.21% over the control) and after watering resumed it recovered slightly. The highest FEGLA was attained in the silking stage in both water regimes (Figure 3) where the water-stressed plants produced 17.64% less FEGLA than well-watered plants (Table 1). During the grain-filling period, the FEGLA reduced gradually

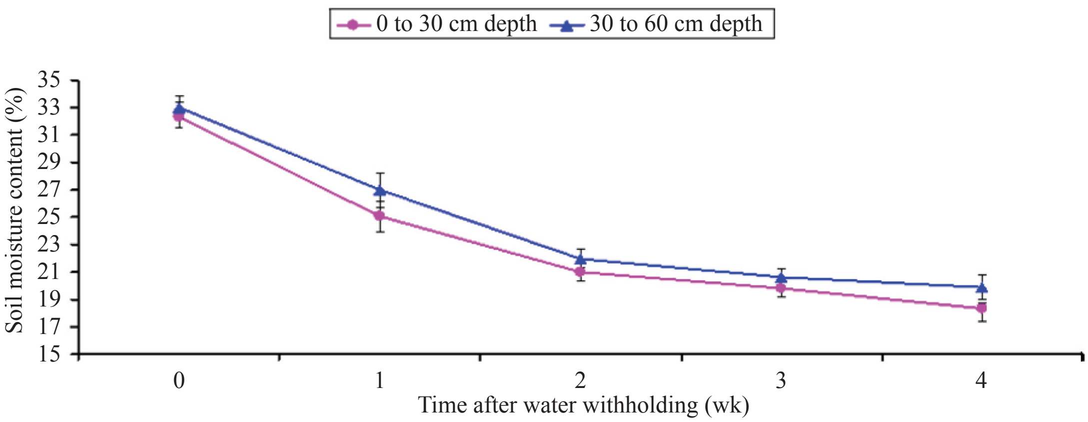

Figure 2 Soil moisture content on the experimental site at 0–30 and 30–60 cm depth after a 30d water withholding period. Error bars show ± SD.

and was more pronounced in the well-watered plants.

Pioneer 30B80 had significantly less FEGLA during the early vegetative stage but from silking onward, there was no significant difference between the two hybrids (Figure 4). Suwan 4452 lost its green leaf portion more rapidly than Pioneer 30B80 being less by 11.57 and 4.62%, respectively, at 17 WAP compared to the silking stage (11 WAP).

Optimal N produced the highest FEGLA level during the experimental period followed by

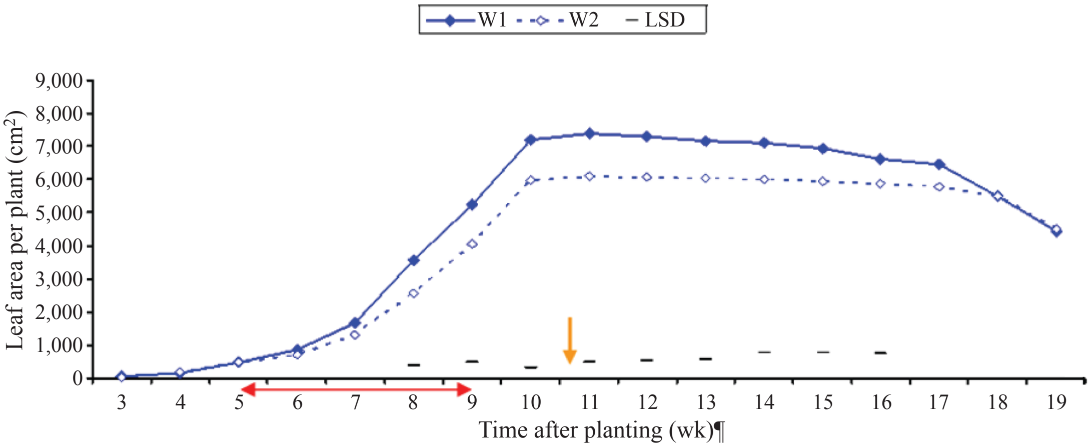

Figure 3 Fully expanded green leaf area kinetics of maize under well-watered (W1) and water-stressed (W2) conditions (Double-headed arrow shows water withholding period; ↓ = Silking stage; LSD = Least significant difference, $P = 0.05$).

Table 1 Main effects of water regime, variety and N level on growth, yield and physiological parameters of maize.

Treatment	Leaf area at silking (cm^2 per plant)	Leaf relative water content (%)	Leaf rolling (score 1-5)	Root dry matter (g per plant)	Grain number (m^{-2})	Grain yield ($g.m^{-2}$)
Water (W)						
Well-watered	7406.60^a	91.08^a	1.00^b	46.41^a	2762.91^a	700.92^a
Water-stressed	6100.29^b	79.36^b	2.09^a	23.75^b	2299.73^b	566.37^b
F– test	**	**	*	**	*	*
Variety (V)						
Pioneer 30B80	6591.20	85.58	1.44^b	48.03^a	2705.90^a	665.80^a
Suwan 4452	6915.69	84.86	1.65^a	22.13^b	2356.74^b	601.49^b
F– test	NS	NS	*	**	**	**
N level (N)						
0 $kg.ha^{-1}$	4929.49^c	84.80	1.90^a	17.80^c	2257.96^c	511.04^c
160 $kg.ha^{-1}$	7933.37^a	85.83	1.27^b	47.44^a	2837.70^a	747.54^a
320 $kg.ha^{-1}$	7397.47^b	85.02	1.47^b	40.01^b	2498.30^b	642.36^b
F– test	**	NS	**	**	**	**
F– test (W × V × N)	NS	NS	**	*	**	*

$^{a–c}$ = Means within a column with the same lowercase superscript are not significant at $P < 0.05$ based on Fisher's least significant difference test. * = Significant at $P < 0.05$; ** = Significant at $P < 0.01$; NS = Not significant.

the supra-optimal and zero N levels, respectively (Figure 5). At the silking stage, optimal N produced 37.86 and 6.76% higher levels of FEGLA than the control and supra-optimal N levels, respectively (Table 1). During the grain-filling period, the FEGLA reduced gradually at all N levels, but was more pronounced in the zero N treatment as this was reduced 11.53% more than the optimal N at 18 WAP compared to 11 WAP.

Only the variety × nitrogen interaction effect (Figure 6) produced significantly different FEGLA values, especially at the silking stage (Table 3). The FEGLA was reduced by 32.33 and 9.88% in Pioneer 30B80 and it was 43.04 and 3.83% in Suwan 4452 with the zero and supra-optimal N levels, respectively, compared to the optimal N at the silking stage (Table 3).

Root dry matter

Averaged across the N levels and varieties, the RDM production was significantly reduced by water stress (48.83%) as shown in Table 1. Pioneer 30B80 showed significantly higher RDM than Suwan 4452 in the surface soil layer averaged over the water regimes and nitrogen levels (Table 1). Averaged across the water regimes and varieties, the N levels had a significant effect on RDM production. Interestingly, the optimal level of N produced significantly higher RDM (15.66%) than the supra-optimal N level whereas

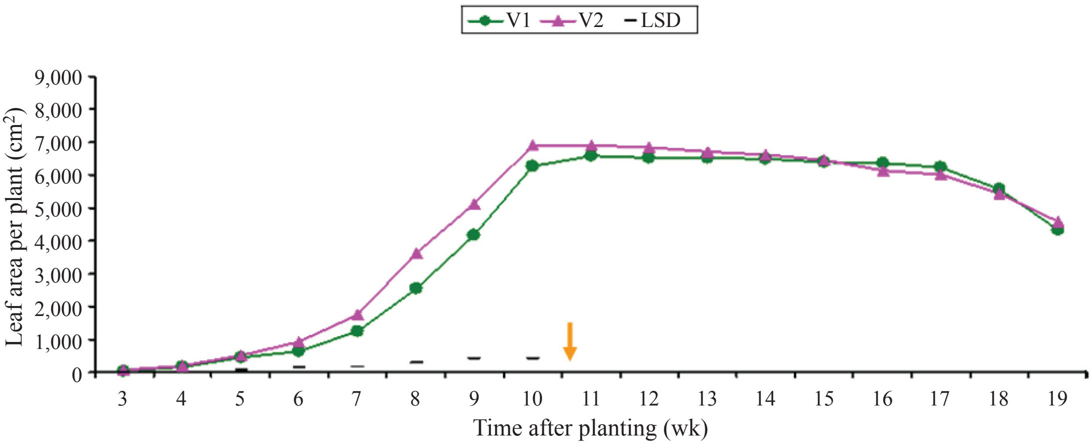

Figure 4 Fully expanded green leaf area kinetics of maize variety (Pioneer 30B80 (V_1) and Suwan 4452 (V_2); ↓ = Silking stage; LSD = Least significant difference, P = 0.05).

Table 2 Effects of water regime × variety interaction on growth, yield and physiological parameters of maize.

Water regime × Variety	Leaf area at silking (cm^2 per plant)	Leaf relative water content (%)	Leaf rolling (score 1–5)	Root dry matter (g per plant)	Grain number (m^{-2})	Grain yield (g.m^{-2})
W1 × V1	7162.72	91.15	1.00^c	64.15^a	2930.01	730.24
W1 × V2	7650.48	91.00	1.00^c	28.68^b	2595.81	671.61
W2 × V1	6019.68	80.01	1.88^b	31.92^b	2481.80	601.36
W2 × V2	6180.90	78.71	2.30^a	15.58^c	2117.67	531.38
F– test	NS	NS	*	**	NS	NS

W1 = Well watered; W2 = Water stressed; V1 = Pioneer 30B80; V2 = Suwan 4452.

$^{a–c}$ = Means within a column with the same lowercase superscript are not significant at P < 0.05 based on Fisher's least significant difference test. * = Significant at P < 0.05; ** = Significant at P < 0.01; NS = Not significant.

zero N produced the lowest (Table 1). Both varieties showed significant losses in RDM due to water stress under the water regime × variety interaction, Pioneer 30B80 produced relatively higher RDM under both water regimes (Table 2). The RDM was also significantly affected by the variety × nitrogen interaction with both hybrids producing lower RDM under both zero and supra-optimal N than optimal N (Table 3). Pioneer 30B80 showed the highest RDM with optimal N while Suwan 4452 had the lowest RDM with zero N. The water regime × nitrogen interaction had a significant influence on RDM; the optimal N level showed better performance in both water regimes (Table 4).

The differences in RDM between the optimal N level and the other levels were relatively lower under the water-stressed conditions than under the well-watered ones. The three-way interaction of water regime × variety × level also had a significant effect on the RDM with Suwan 4452 having the lowest RDM under combined stress.

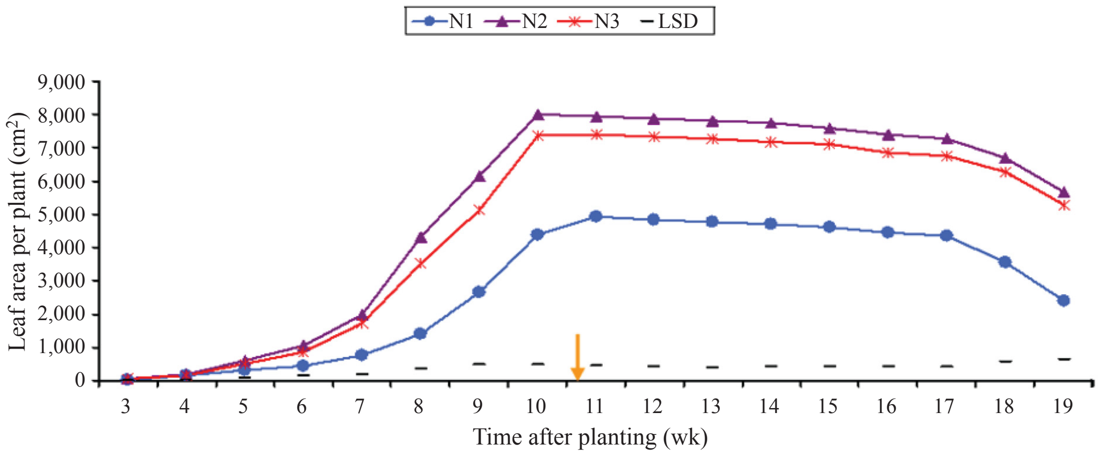

Figure 5 Fully expanded green leaf area kinetics of maize under different N levels: 0 $kg.ha^{-1}$ (N1), 160 $kg.ha^{-1}$ (N2) and 320 $kg.ha^{-1}$ (N3). (↓ =Silking stage; LSD = Least significant difference, P = 0.05).

Table 3 Effects of variety × nitrogen interaction on growth, yield and physiological parameters of maize.

Variety × Nitrogen	Leaf area at silking (cm^2 per plant)	Leaf relative water content (%)	Leaf rolling (score 1-5)	Root dry matter (g per plant)	Grain number (m^{-2})	Grain yield ($g.m^{-2}$)
V1 × N1	5190.80^c	85.12	1.62^b	24.51^c	2542.64^c	586.29^d
V1 × N2	7670.49^a	86.21	1.18^c	65.03^a	3017.33^a	782.61^a
V1 × N3	6912.31^b	85.42	1.52^{bc}	54.56^b	2557.73^c	628.50^c
V2 × N1	4668.18^c	84.49	2.18^a	11.09^d	1973.28^e	435.78^e
V2 × N2	8196.26^a	85.46	1.35^c	29.84^c	2658.08^b	712.47^b
V2 × N3	7882.63^a	84.62	1.42^{bc}	25.46^c	2438.87^d	656.22^c
F– test	**	NS	**	**	**	**

V1 = Pioneer 30B80; V2 = Suwan 4452; N1 = 0 $kg.ha^{-1}$, N2 = 160 $kg.ha^{-1}$; N3 = 320 $kg.ha^{-1}$.

$^{a–e}$ = Means within a column with the same lowercase superscript are not significant at $P < 0.05$ based on Fisher's least significant difference test. * = Significant at $P < 0.05$; ** = Significant at $P < 0.01$; NS = Not significant.

Relative water content and leaf rolling

The RWC was significantly affected only by the main effect of water regime and the reduction of RWC was 12.87% due to water stress (Table 1). LR was significantly affected by all the main and interaction effects at the end of the water stress period (Tables 1–4). The leaf rolling scores differed clearly by variety and water regime separately, and in the case of N level, there was a difference only in the zero N level (Table 1). The other interaction result differences were more pronounced when one or more of Suwan 4452, zero N and the water-stressed component were present.

Grain number and grain yield

The main effects of water regime, variety and nitrogen level had a significant influence on the GNM and GY (Table 1). Among the interaction effects, only the variety × N level and water × variety × N level had significant effects on the GNM and GY (Table 3 and Figure 7). The

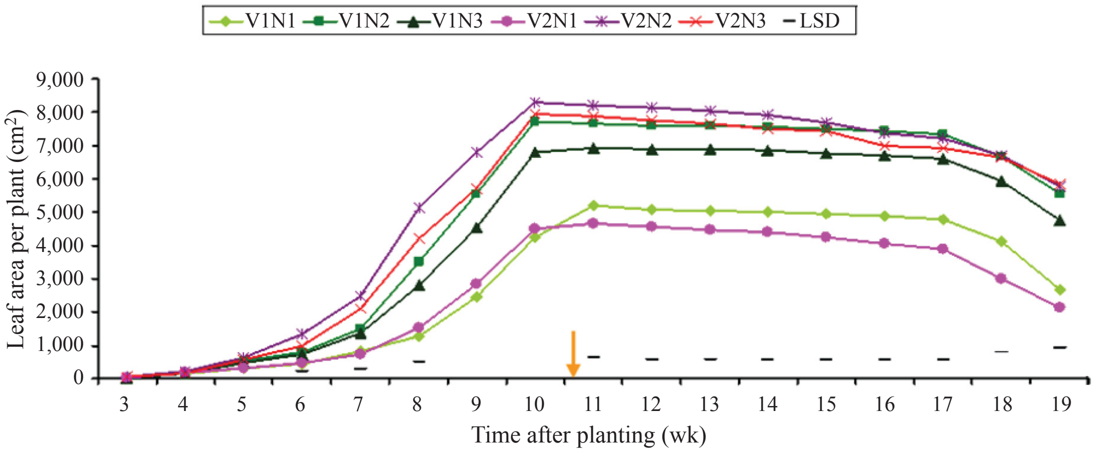

Figure 6 Fully expanded green leaf area kinetics of maize as affected by variety × nitrogen level interaction (Variety = Pioneer 30B80 (V1) and Suwan 4452 (V2); Nitrogen level = 0 kg.ha^{-1} (N1), 160 kg.ha^{-1} (N2) and 320 kg.ha^{-1} (N3); ↓ = Silking stage; LSD = Least significance difference, P = 0.05).

Table 4 Effects of water regime × nitrogen interaction on growth, yield and physiological parameter of maize.

Water regime × Nitrogen	Leaf area at silking (cm^2 per plant)	Leaf relative water content (%)	Leaf rolling (score 1-5)	Root dry matter (g per plant)	Grain number (m^{-2})	Grain yield (g.m^{-2})
W1 × N1	5742.48	90.90	1.00^d	23.06^d	2530.68	581.23
W1 × N2	8509.05	91.80	1.00^d	64.85^a	3068.88	820.56
W1 × N3	7968.26	90.54	1.00^d	51.34^b	2689.16	700.97
W2 × N1	4116.50	78.71	2.80^a	12.55^e	1985.24	440.84
W2 × N2	7357.70	79.87	1.53^c	30.02^c	2606.53	674.52
W2 × N3	6826.68	79.50	1.93^b	28.68^{cd}	2307.44	583.74
F– test	NS	NS	**	**	NS	NS

W1 = Pioneer 30B80; W2 = Suwan 4452; N1 = 0 kg.ha^{-1}; N2 = 160 kg.ha^{-1}; N3 = 320 kg.ha^{-1}.
$^{a–e}$ = Means within a column with the same lowercase superscript are not significant at P < 0.05 based on Fisher's least significant difference test. * = Significant at P < 0.05; ** = Significant at P < 0.01; NS = Not significant.

GNM and GY were reduced 16.76 and 19.20%, respectively, due to the main effect of water stress where the reduction was 12.90 and 9.66%, respectively, in Suwan 4452 compared to Pioneer 30B80 (Table 1). Under the main effect, the optimal level of N had significantly higher levels of GNM (11.96%) and GY (14.07%) than the supra-optimal N level whereas zero N produced the lowest GNM and GY.

Considering interaction effects, Pioneer 30B80 produced significantly the highest GNM and GY values with the optimal level of N; the GNM of Pioneer 30B80 at the zero N level was similar to the other treatments except for the Suwan 4452 × zero N treatment which had the lowest GNM and GY (Table 3). Both hybrids produced significantly the highest GNM and GY under the optimal conditions and Suwan 4452 produced the lowest under combined stress (Figure 7).

DISCUSSION

Among the plant growth processes, leaf elongation is the most sensitive to water deficit (Bogoslavsky and Neumann, 1998). Averaged across the N levels and varieties, the FEGLA of pre-anthesis drought-stressed maize reduced sharply and after re-watering it recovered slightly (Mansouri-Far *et al.*, 2010) but not fully because the remaining non-expanded leaves only could be expanded under well-watered conditions. Typically, the leaf expansion of maize is rapid during 30 to 49 DAP (Tollenaar and Dwyer, 1999) and water stress around this period affects the leaf development and expansion (Passioura *et al.,* 1993). As a result, the leaf area was reduced which might have resulted from a reduced supply of nutrient from the soil due to the unavailability of nutrients (Pandey *et al.*, 2000b and Mansouri-Far *et al.*, 2010) and less root proliferation (Table 1). Abscisic acid (ABA) signaling and stomatal closing which hampered photosynthesis might have been other major causes of the reduced leaf area under water-stressed conditions (Zhang and Davies, 1990). During the whole grain filling period, well-watered plants lost more green leaf area from senescence and this might have been due to the greater translocation of nitrogen to the ear as it created a higher sink than in water-stressed plants during the vegetative stage. The FEGLA was also different due to varietal difference—leaf appearance in Pioneer 30B80 was relatively slower than in Suwan 4452—and from full leaf appearance onward, there was no significant difference in the FEGLA, with Pioneer 30B80 being the relatively greener variety. During the grain filling stage, stay-green genotypes can take up N in a greater portion of leaves because the continued leaf activity promotes the uptake of soil

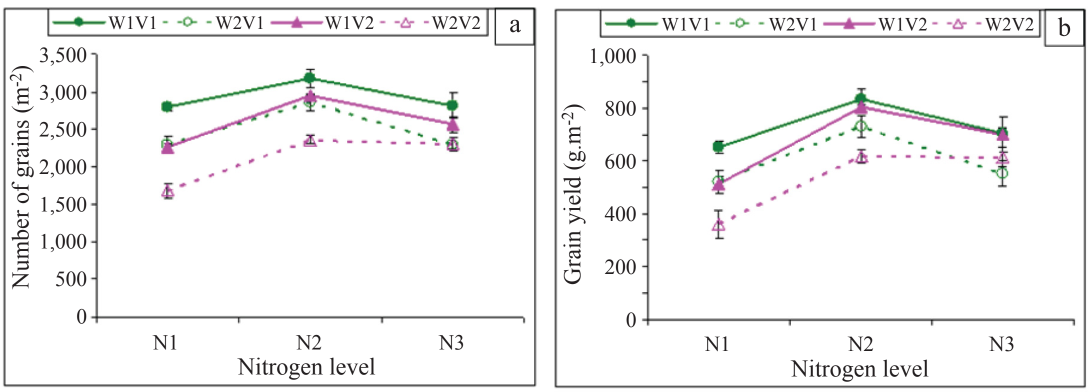

Figure 7 Interaction effects of water regime, N level (N1 = 0 kg.ha^{-1}; N2 = 160 kg.ha^{-1}; N3 = 320 kg.ha^{-1}) and variety on: (a) Grain number and (b) Grain yield at final harvest of maize. Error bars show ± SD.

N (Woodruff, 1972). Though the total leaf area of Pioneer 30B80 was relatively lower than in Suwan 4452, the former could produce a greater grain yield, because, it could maintain a leaf area index > 4 during the whole grain filling period. It has been reported that radiation interception increases hyperbolically up to a leaf area index of 3–4, after which, it is little affected by further increases in the leaf area index (Muchow, 1988). The reduced leaf area in the zero N level treatment was mainly due to a shortage of N supplied from the soil, which is required to maintain the proper specific N level in the leaves (Muchow, 1988), which in turn could reduce the amount of leaf chlorophyll followed by less photosynthesis and photoassimilation (Pandey *et al.*, 2000b). With the supra-optimal N level, the FEGLA was also reduced, perhaps due to lower root proliferation (Table 1), which caused a limited uptake of essential nutrients from the soil. It might also have been due to an excess amount of NH_4^+ N penetration through the roots a few days just after the two urea applications, which might have triggered a higher pH (Hoffmann and Kosegarten, 1995) leading to ABA signaling (Morgan, 1986; Liu and Dickmann, 1992) and substitution of K^+ followed by stomatal closure and reduced photosynthesis. There was no significant difference in the FEGLA between the maize varieties at a certain level of N application (Table 3) and also none due to the interaction between the N level and water stressing (Table 4) which agreed with Mansouri-Far *et al.* (2010). According to Wilkinson *et al.* (2007), high N reduced the stomatal conductance and transpiration in dry soil, whilst the leaf area remained higher than in zero N plants (Morgan, 1986). It seemed that Pioneer 30B80 was relatively more efficient regarding N uptake and lesser susceptible to zero N than Suwan 4452, as the former showed similar performance under combined stresses which might have been due to the greater RDM production under all conditions.

Pioneer 30B80 was relatively less susceptible to pre-anthesis drought than Suwan 4452 and it might have been due to the relatively higher volume of root production in the surface soil during the early vegetative stage, which was also supported by Oikeh *et al.* (1999). Eghball *et al.* (1993) also reported that N stress resulted in few roots branching in maize, which would involve an impaired ability to take up nutrients. Low photoassimilates production and translocation to the roots under the zero nitrogen condition might have been a constraint on proper root growth. Optimal N may have been conductive to greater root development (Pandey *et al.* 2000b) and an excessive application inhibited root growth in maize (Wang *et al.*, 2008). This inhibition might occur due to several causes, such as: toxicity resulting from excess N (Wang *et al.*, 2008) and raising the soil pH for the first few days just after urea application when excess NH_4^+ is produced (Singh and Yadav, 1981) that hampers the uptake of some essential nutrients (Britto and Kronzucker, 2002). An additional resosn could be the penetration of excess NH_4^+ through the roots might depress K^+ and be accompanied by an increase in chloride (Britto and Kronzucker, 2002) or there could be an increase in sap pH (Hoffmann and Kosegarten, 1995) causing ABA signaling and finally there could be hampering of the photoassimilate production through stomatal closure (Liu and Dickmann, 1992; Morgan, 1986). Excess NO_3 availability surrounding the root zone might also discourage root growth (Mi *et al.*, 2008).

Other studies have reported similar results to those from the present study regarding leaf RWC being more or less similar within the varieties (Mansouri-Far *et al.*, 2010) or with N levels (Mansouri-Far *et al.*, 2010; Wilkinson *et al.*, 2007), but differing significantly under water stress, (Mansouri-Far *et al.*, 2010). Under water-stressed conditions in clay soil, Pioneer 30B80 retained more than 80% RWC, which was in the acceptable range as per the suggestion of Obeng-Bio *et al.* (2011). This reduction might have been due to a decrease in root permeability caused by the higher bulk density and anaerobic conditions in the lower soil layer (Kar and Verma, 2005).

According to Obeng-Bio *et al.* (2011), genotypes with LR indices greater than 3 might be susceptible to drought, as such leaf rolling could inhibit full photosynthetic capacity and dry matter production. Although the LR scores differed significantly due to all the main and interaction effects, most of the scores were in the acceptable range except for Suwan 4452 under combined stress. However, the optimal N application had a positive effect on retaining more RWC and maintaining turgidity whilst high N reduced the shoot water potential in dry soil in several cases (Morgan, 1986).

According to Mueller and Pope (2009), GNM is the most important attribute for grain yield, which is determined during vegetative growth (6–12 leaf stage) and might have been for this reason in the present study that there was a decrease in the total number of potential ovules per ear that was reflected in the final GNM and GY values. Pioneer 30B80 is a semi-prolific variety (data not shown) and produces more GNM and GY than Suwan 4452 which was also supported by Kamprath *et al.* (1982). During the vegetative stage of maize, N availability is very crucial in determining the potential ovule formation (Mueller and Pope, 2009) and in the present study, plants supplied with the zero N level produced lower GNM and subsequently lower GY. In the case of the supra-optimal N level, the small GNM and GY might have been due to less nutrient uptake through the weak root system and less photo assimilates produced from the limited FEGLA. Uribelarrea *et al.* (2007) suggested that both the variety and N rate affected plant N accumulation and uptake efficiency. However, Pioneer 30B80 was relatively less susceptible to the zero N level but relatively more sensitive to the supra-optimal N level, where it produced relatively less RDM, GNM and GY. This partially supported the suggestion by Beauchamp *et al.* (1976) and Kamprath *et al.* (1982) that cultivars differ in their ability to absorb and utilize N from the soil. The observed variation in susceptibility to water and N stress among varieties suggested that the traits can be improved.

CONCLUSION

A significant varietal difference was found regarding growth, physiology and grain yield, although only two varieties were tested and so did not represent a broad sample of tropical germplasm. Stress tolerance characteristics could be considered to be maintaining greater root volume, staying green, showing prolificacy in grain number and having a RWC higher under both nitrogen and water-stressed conditions. Optimal levels of N fertilizer always showed better performance regardless of the variety or nitrogen level or whether there was water stress. Therefore, the application of N fertilizer at optimal levels can reduce the fertilizer application rate, which will be helpful for the reduction of environmental pollution and the cost of agricultural practices.

ACKNOWLEDGEMENTS

The authors express their appreciation to Mr. Surapol Chowchong and the staff of the National Corn and Sorghum Research Center, Nakhon Ratchasima province, Thailand for their support and assistance in carrying out the field trials. This project was conducted in collaboration with the Faculty of Agriculture, Kasetsart University, Bangkok and was financially supported by National Agricultural Technology Project (NATP), Phase-1, Bangladesh Agricultural Research Council (BARC).

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