

## EXPLORING OPTIMIZATION OF WATER AND NITROGEN FERTILIZER MANAGEMENT FOR POTTED MAIZE BASED ON PCA

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### Abstract

Deficit irrigation has been widely used in crop production, but there is not enough information about how and whether water stress and nitrogen (N) fertilizer interact with maize (*Zea mays* L.) growth and nitrogen uptake in pot experiments. In this study, the effects of deficit irrigation and N fertilizer application rate at different growth stages on physiological and morphological parameters of potted maize were studied to determine whether the jointing stage was the key period of maize water demand. The experiment was arranged in a randomized complete block design with two factors (5 irrigation levels  $\times$  4 N fertilizer application rates) and replicated three times. Leaf area, plant height, root length, root activity, dry matter accumulation, and N uptake decreased with the increase of N fertilizer application rates. The plant height in deficit irrigation at mature stage ( $D_m$ ) was the highest, which was 9.4% ~ 20.5% higher than that of deficit irrigation at other growth stages. Deficit irrigation at filling stage ( $D_f$ ) had the highest chlorophyll content, which was 3.6% ~ 9.4% higher than that of other irrigation treatments on averaging N fertilizer application rates. The root number increases first and then decreases with the increase of N fertilizer application rate on averaging irrigation treatments. The mean water use efficiency (WUE) order from high to low was  $D_s$  (deficit irrigation at seedling stage)  $>$   $D_m$   $>$   $F_i$  (full irrigation)  $>$   $D_f$   $>$   $D_j$  (deficit irrigation at jointing stage) on averaging N fertilizer application rates. WUE was only significant correlated with plant height. The NUE was significantly correlated with leaf area, chlorophyll content, root activity, dry matter accumulation and nitrogen uptake. The results showed that the jointing stage was sensitive to water and nitrogen requirements. The  $F_iN_1$  (full irrigation, and 0.1 g N  $kg^{-1}$  soil) treatment ranked first after analyses in the combinational evaluation, which provides a reference for evaluating and selecting better field crop management methods.

**Key words:** Maize; Leaf area; Dry matter accumulation; Root activity; Water use efficiency; Nitrogen use efficiency, PCA.

### Introduction

Soil water content and soil nitrogen (N) availability are two important factors that influence the crop growth and production (Gautam *et al.*, 2008; Mitchell *et al.*, 2000). Maize is a crop of high-water and high-N consumption, and the input of water and N directly influences the dry matter accumulation and yield formation (Adamtey *et al.*, 2010; Echarte *et al.*, 2008). Many researchers have conducted experiments of deficit irrigation techniques on vegetable, ornamental plants and field crops (Dacosta & Huang, 2006; Du *et al.*, 2010; El-Mageed & Semida, 2015; Igbadun *et al.*, 2008; Kloss *et al.*, 2014; Yonts *et al.*, 2018), and the results indicated that deficit irrigation can significantly improve water use efficiency (WUE). Furthermore, the grain yield of maize showed no significant difference in moderate deficit irrigation than full irrigation under the particular maize varieties (Kaman *et al.*, 2011). Moderate deficit irrigation only showed a slightly reduced plant height and total dry weight, and the flowers number and colour parameters have no obvious difference and statistically significant with that of full irrigation in potted Carnations (Álvarez *et al.*, 2009). However, the severe deficit irrigation was significantly decreased grain yield, even more serious, which induced higher leaf pressure values and cannot meet the normal growth of maize (Mansouri-Far & Saberali, 2010; Yin *et al.*, 2016). The time of deficit irrigation was most important to plant growth and quality (Álvarez *et al.*, 2013), this demonstrates that crop growth does not only depend on the irrigation amount supplied but also on the time when the deficit irrigation is applied, and that flowering is the most sensitive

period to water stress (Ma *et al.*, 2012; Rodrigues *et al.*, 2013). So, the irrigation water requirements and sensitivity phase to water deficits of maize is of great importance to agronomists for planning irrigation strategies. Some researchers have carried out the field experiment and confirmed that tasseling stage is the critical period of water demand in maize crops, followed by jointing stage (Hall *et al.*, 1982; Yang *et al.*, 2017). Meanwhile, the grain yield showed decreased with water stress, and the more serious of water stress the more significant influence of the N-fertilizer rate (Castronava *et al.*, 2014; Pandey *et al.*, 2000).

Many researchers focus on coupling effects of deficit irrigation and N-fertilizer levels on maize growth and yield, which is beneficial to determine the indicators for precision agriculture (Han *et al.*, 2013; Pandey *et al.*, 2000). The interactive influences of irrigation and N-fertilizer was significantly effect on nitrate loss, which is the most effective measure for reducing nitrate leaching out of the root zone under a proper combination of water-fertilizer management (Gheysari *et al.*, 2009; Wang *et al.*, 2019a). There was a synergistic effect of irrigation and N-fertilizer on maize yield, and a propriate water-fertilizer management can both obtain a relatively higher grain yield and large economic benefit (Han *et al.*, 2013, Prunty & Montgomery, 1991). The optimal amounts of irrigation and N-fertilizer is important for field management, the water resources pollution by N and other nutrient elements becomes a byproduct when the fertilization input exceeds the crop consumption (Jia *et al.*, 2014; Wang *et al.*, 2019b). These high-fertilizer inputs and the extremely low crop recoveries of fertilizer nutrients have caused the marked deterioration of soil and groundwater

quality. In recent years, researchers and public figures have increased their concerns on the protection of water resource from pollution attribute to N from agricultural sources (Godfray *et al.*, 2010; Wang *et al.*, 2015).

In addition, when the irrigation amount applied during whole growth stages does not meet the crop evapotranspiration demand, the N-fertilizer application rate based on calculations for full irrigation may cause an unobservant over-application of N, which could increase the potential for low nitrogen use efficiency and environmental impact of future N losses to the groundwater.

However, the requirement of water was different in different development stage of maize crops (Çakir, 2004; Fan *et al.*, 2020). In arid regions, the main limiting factors for maize production is the water shortages. Deficit irrigation is the normal water saving irrigation measures in these areas. Although deficit irrigation might reduce crop yield to a certain degree, the quality can improve in the meantime. N-fertilizer is able to implement such the functionality that neutralizes the water stress on yields and has a positive impact on crop growth if the deficit irrigation is appropriate levels and appropriate time. In sensitive period of water and N-fertilizer requirement, temporary water stresses and N shortage have significant influences on maize growth, as well as the fresh and dry matter accumulations of the whole plants. So, it is very important and extremely urgent to optimize a water and N-fertilizer management strategy for maize grown to balance the input of water and N-fertilizer resources and maize growth. In this study, we carried out a pot maize experiment tries to (1) investigate the coupling effects of deficit irrigation at different development stages and N-fertilizer application levels on maize growth and N uptake and (2) assess the sensitive period of water and N-fertilizer requirement in maize growth by principal component analysis (PCA) in a calcareous soil. Answering these questions is important for selecting better field management techniques and providing better ideas to reduce N-fertilizer overuse in calcareous soils.

## Materials and Methods

**Study site description:** The potted experiment was performed at Ansai County, Shaanxi province, China, located at 36°39' N and 109°11' E. The experiment was performed between May and September 2017, in a rain shelter belongs to Yan'an University. The experimental sites have an altitude of 1100 m. The annual average air temperature is 8.7°C and annual average precipitation is 531 mm (from 1952 to 2015). Calcareous soil was used (Pan *et al.*, 2017), with details on its physical and chemical character as follows: soil pH, 8.2; soil bulk density, 1.3 g cm<sup>-3</sup>; soil organic matter content, 12.3 g kg<sup>-1</sup>; soil total N content, 0.83 g kg<sup>-1</sup>; soil available phosphorus content, 11.8 mg kg<sup>-1</sup>; soil available potassium content, 78.2 mg kg<sup>-1</sup>; soil field capacity ( $\theta_f$ , vol%), 24.1%; permanent wilting point 12.5%.

**Experimental design:** The maize was planted in the plastic bucket. The above diameter of plastic bucket is

28.5-cm, the below diameter is 21-cm, and the height is 24.5-cm. Nine holes was designed for a plum blossom-like arrangement by artificial in the bottom of the bucket so as to provide good ventilation conditions. Meanwhile, the bottom of bucket covered with 80 mesh nylon and fine sand is effectively for prevention water and nutrient loss. The topsoil (in the plough layer of 20–50 cm) in the farmland was used as soil samples in this experiment. The selected soil was air dry in natural conditions, and then sieved the air-dried soils through 5 mm sieve.

The experiment was arranged with two factors (5 irrigation levels  $\times$  4 N-fertilizer application rates) and three replicates. Five irrigation treatments were assessed: (1) full irrigation (100% $\theta_f$ , where  $\theta_f$  is the field capacity) in the whole growth stage ( $F_i$ ); (2) deficit irrigation (65–80% $\theta_f$ ) was only applied in the seedling stage ( $D_s$ , from 10 to 24 days after planting); (3) deficit irrigation (65–80% $\theta_f$ ) was only applied in the jointing stage ( $D_j$ , from 25 to 48 days after planting); (4) deficit irrigation (65–80% $\theta_f$ ) was only applied in the filling stage ( $D_f$ , from 49 to 70 days after planting); (5) deficit irrigation (65–80% $\theta_f$ ) was only applied in the maturity stage ( $D_m$ , from 71 to 89 days after planting). Four N-fertilizer application rates were assessed: (1) no N-fertilizer applied ( $N_0$ ); (2) N-fertilizer applied as 0.1 g N kg<sup>-1</sup> soil ( $N_1$ ); (3) N-fertilizer applied as 0.3 g N kg<sup>-1</sup> soil ( $N_2$ ); (4) N-fertilizer applied as 0.5 g N kg<sup>-1</sup> soil ( $N_3$ ). The N-fertilizer was used the analysis pure-N of urea and all the treatments were applied 0.2 g KH<sub>2</sub>PO<sub>4</sub> kg<sup>-1</sup> soil (same phosphate (P) and potassium (K) fertilizer application rate).

The maize breed (*Zea mays* L., cv. 'Dafeng 26' a local variety) was sown on 12<sup>th</sup> May 2017. Before sowing, each treatment received the total amount of N-, P- and K-fertilizer, which were applied at the soil and mixed until well combined. Each plot added finished product soil samples of 15 kilograms and controlled the soil bulk density of 1.3 g cm<sup>-3</sup>. The surface is added to vermiculite (10 g per pot) that in order to keep soil from packing together and reduce surface soil moisture loss. After all preparations have been completed, two maize seeds were planted at the depth of 3-cm. Almost immediately all the treatments irrigated to field capacity on behalf of ensure the moisture needed for germination. The determination of irrigation amount is adopted by weighing method. The irrigation was carried out at soil water content reduces to or approximate to low irrigation limit, and the irrigation amount was controlled at the upper irrigation limit of field capacity. The graduated cylinder was used to measuring the required amount of irrigation in each time, and the total irrigation amount was determined by the added irrigation amount in each time.

**Sampling and measurement:** During the whole growing season, the leaf area, plant height, chlorophyll content, root number, root length, root activity, dry matter accumulation and N uptake were measured four times. The four measurement times were at the end of seedling stage (24 days after planting), jointing stage (48 days after planting), filling stage (70 days after planting) and maturity stage (89 days after planting), respectively.

- (1) The leaf area (LA, cm<sup>2</sup>) was measured by measuring rule and used the leaf area calculation formula (Amanullah *et al.*, 2009; Wang *et al.*, 2014):

$$LA = L \times W \times 0.75 \quad (1)$$

where L is the leaf length (cm) and W is the leaf width (cm), and the leaf area per plant was sum of each leaf area.

- (2) The plant height (cm) was measured by measuring rule.  
 (3) The chlorophyll content was measured by SPAD-502 chlorophyll meter (Korkovelos & Goulas, 2011).  
 (4) The root number and root length were measured by the root samples in each treatment were scanned with a scanner (Epson Perfection v700 Photo, EPSON Company, Japan).  
 (5) The root activity was measured using triphenyltetrazolium chloride (TTC) (Hawrylak-Nowak *et al.*, 2015).  
 (6) The two sub-samples of roots and aboveground biomass from the same treatments were pooled to form a composite roots and aboveground sample. Before weighing, all root and aboveground samples were oven dried at 75°C to constant weight.  
 (7) The N concentration (mg g<sup>-1</sup>) in roots and aboveground were determined by a modified Kjeldahl digestion method (Xiao *et al.*, 2017), and the N uptake (mg plant<sup>-1</sup>) equal to roots and aboveground N concentration multiply by roots and aboveground biomass, respectively.  
 (8) The water use efficiency (WUE, g L<sup>-1</sup>) was calculated using the following formula:

$$WUE = \frac{DMA}{IW} \quad (2)$$

where DMA is the roots and aboveground biomass (g), and IW is sum of irrigation amount (L).

- (9) The nitrogen use efficiency (NUE, %) was calculated based on the following formula:

$$NUE = \frac{N_{\text{uptake}} - N_{\text{uptake},0}}{N_{\text{application}}} \times 100 \quad (3)$$

where  $N_{\text{uptake}}$  is the N uptake amount per plant in N application treatment (g plant);  $N_{\text{uptake},0}$  is the N uptake amount per plant with no N-fertilizer applied treatment (g plant<sup>-1</sup>);  $N_{\text{application}}$  is the N-fertilizer application rate per plant (g plant<sup>-1</sup>).

**Statistical analysis:** Two-way analysis of general linear model (GLM, univariate with equal variances assumed, LSD tests at the significance level of 0.05), with irrigation and N-fertilizer as the two fixed factors, was used to assess variations in the leaf area, plant height, chlorophyll content, root number, root length, root activity, dry matter accumulation, N uptake, WUE and NUE. The mean comparisons were detected using One-way ANOVA (Tukey's-b tests at the significance level of 0.05, SPSS

software, 16.0, SPSS Inst., USA). The correlation among the all parameters used Pearson as correlation coefficients (two-tailed). PCA was used to analyze all the mean parameter values at the maturity stage for different irrigation and N-fertilizer treatments.

## Results

**Leaf area:** The deficit irrigation significantly affected the leaf area during seedling and jointing stage, and the N-fertilizer significantly ( $p < 0.01$ ) affected the leaf area during jointing, filling and maturity stage, however, the interactions between these factors only had a significant effect on leaf area at filling stage (Table 1). At seedling stage, the mean leaf area was higher in D<sub>s</sub> (211 cm<sup>2</sup>) than F<sub>i</sub> (183 cm<sup>2</sup>) on averaging the N-fertilizer application rates (Fig. 1a). At jointing stage, the influence degree of deficit irrigation on leaf area was increased as the growth period progresses that the mean leaf area was higher in F<sub>i</sub> (2796 cm<sup>2</sup>), which was 18.7% higher than D<sub>s</sub> and 15.7% higher than D<sub>j</sub> on averaging N-fertilizer application rates (Fig. 1b). The negative correlation between N-fertilizer application rate and leaf area, meaning the higher the N-fertilizer applied, the lower the leaf area at jointing stage (Fig. 1b).

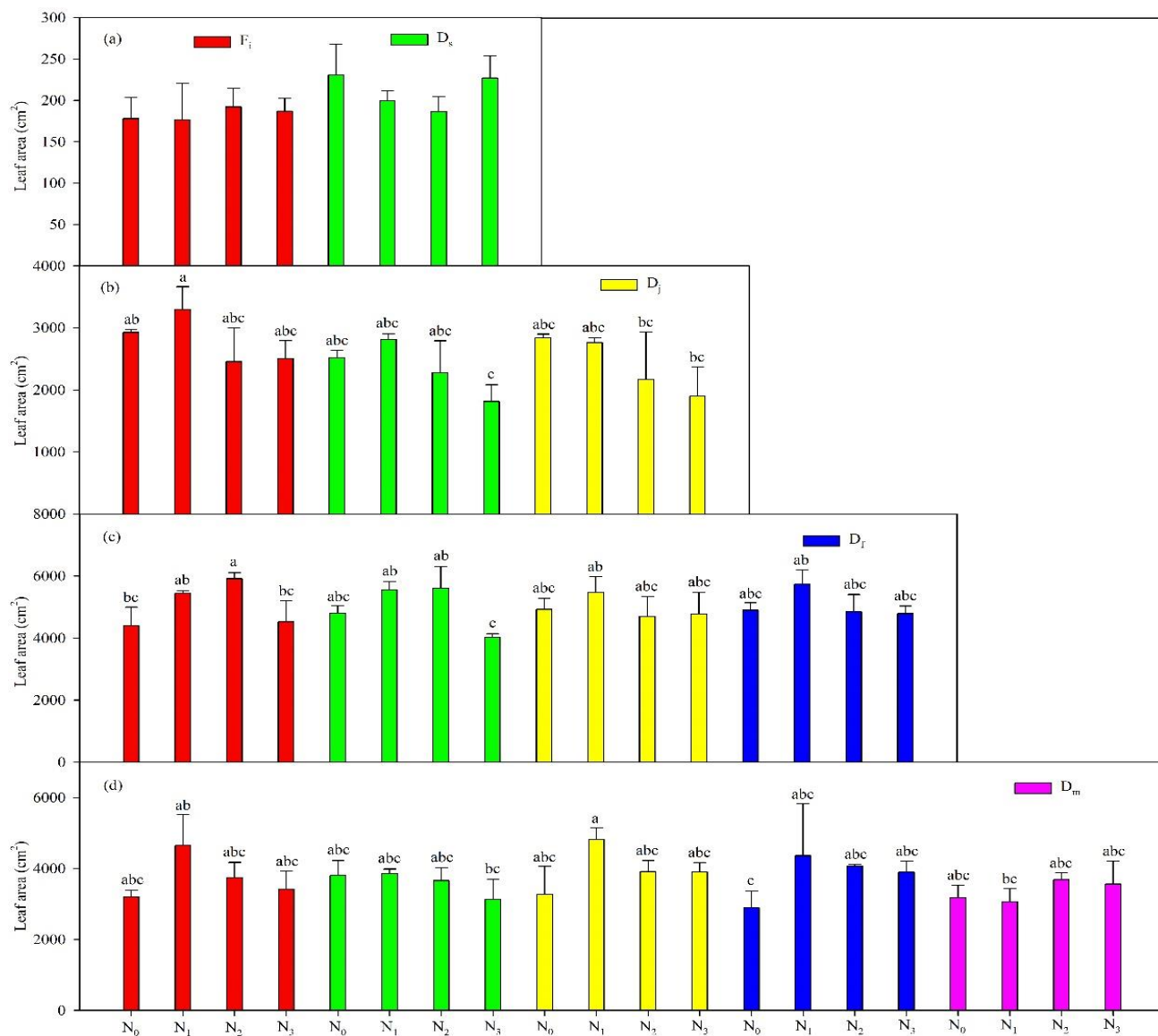
At filling stage, the leaf area was also decreased with the increase of N-fertilizer application rate, the mean leaf area was higher in N<sub>1</sub> (5548 cm<sup>2</sup>) treatment, which was 5.3% (279 cm<sup>2</sup>) and 22.5% (1019 cm<sup>2</sup>) higher than N<sub>2</sub> and N<sub>3</sub> on averaging irrigation treatments, respectively (Fig. 1c). On averaging N-fertilizer application rates, the order of leaf area from high to low are F<sub>i</sub> > D<sub>f</sub> > D<sub>s</sub> > D<sub>j</sub> at filling stage (Fig. 1c). At maturity stage, the mean leaf area in D<sub>j</sub> (3974 cm<sup>2</sup>) and D<sub>f</sub> (3806 cm<sup>2</sup>) were higher than F<sub>i</sub> (3753 cm<sup>2</sup>) on averaging N-fertilizer application rates, and the follows that D<sub>s</sub> (3615 cm<sup>2</sup>) and D<sub>m</sub> (3371 cm<sup>2</sup>) (Fig. 1d). On averaging irrigation treatments, the mean leaf area was also decreased with the increase of N-fertilizer application rate, the mean leaf area was higher in N<sub>1</sub> (5189 cm<sup>2</sup>) treatment, which was 8.9% (422 cm<sup>2</sup>) and 15.9% (711 cm<sup>2</sup>) higher than N<sub>2</sub> and N<sub>3</sub> at maturity stage, respectively (Fig. 1d).

**Plant height:** The individual factor of irrigation and N-fertilizer significantly ( $p < 0.01$ ) affected the plant height during seedling, jointing and maturity stage, and the interactions between these factors had a significant ( $p < 0.01$ ) effect on plant height at maturity stage (Table 1). At seedling stage, the mean plant height was higher in F<sub>i</sub> (64 cm) than D<sub>s</sub> (59 cm) on averaging the N-fertilizer application rates (Fig. 2a). The mean plant height was decreased with the increase of N-fertilizer application rate, and the value in N<sub>1</sub> (62 cm) was 2.6% (2 cm) and 11.6% (7 cm) higher than N<sub>2</sub> and N<sub>3</sub> at seedling stage, respectively (Fig. 2a). At jointing stage, the mean plant height was lower in D<sub>j</sub> (124 cm), which was 9.9% (12 cm) and 10.7% (13 cm) lower than D<sub>s</sub> and F<sub>i</sub> on averaging N-fertilizer application rates, respectively (Fig. 2b). The plant height was decreased with the increase of N-fertilizer application rate on averaging irrigation treatments at jointing stage, and mean plant height in N<sub>1</sub> was 7.6% (15 cm) and 11.7% (22 cm) higher than N<sub>2</sub> and N<sub>3</sub>, respectively (Fig. 2b).

**Table 1. Treatment effects (P values) on leaf area (LA), plant height (PH), chlorophyll content (CC), root number (RN), root length (RL), root activity (RA), dry matter accumulation (DMA), nitrogen uptake (NU) in seedling stage (SS), jointing stage (JS), filling stage (FS) and maturity stage (MS), using irrigation (I) and nitrogen fertilizer (N) and irrigation (W) as two fixed factors.**

Stage	Factors	LA	PH	CC	RN	RL	RA	DMA	NU
SS	I	*	**	ns	ns	ns	**	ns	**
	N	ns	**	*	**	**	**	**	**
	I × N	ns	ns	ns	ns	ns	ns	ns	**
JS	I	*	**	ns	**	ns	**	*	**
	N	**	**	*	**	*	**	*	**
	I × N	ns	ns	ns	ns	ns	ns	ns	**
FS	I	ns	ns	ns	**	ns	**	ns	**
	N	**	ns	**	**	**	**	**	**
	I × N	**	ns	ns	ns	ns	ns	ns	**
MS	I	ns	**	ns	**	ns	**	ns	**
	N	**	**	**	**	**	**	**	**
	I × N	ns	**	ns	ns	ns	**	ns	**

Note: “\*\*\*” means  $p < 0.01$ , “\*\*” means  $0.01 < p < 0.05$  and “ns” means  $p > 0.05$



**Fig. 1. Leaf area per plant were affected by irrigation treatment (F<sub>i</sub>, full irrigation in the whole growth stage; D<sub>s</sub>, deficit irrigation was only applied in the seedling stage; D<sub>j</sub>, deficit irrigation was only applied in the jointing stage; D<sub>f</sub>, deficit irrigation was only applied in the filling stage; D<sub>m</sub>, deficit irrigation was only applied in the maturity stage) and N-fertilizer application rates (N<sub>0</sub>, no N-fertilizer applied; N<sub>1</sub>, N-fertilizer applied as 0.1 g N kg<sup>-1</sup> soil; N<sub>2</sub>, N-fertilizer applied as 0.3 g N kg<sup>-1</sup> soil; N<sub>3</sub>, N-fertilizer applied as 0.5 g N kg<sup>-1</sup> soil) in seedling stage (a), jointing stage (b), filling stage (c) and maturity stage (d). Bars are the means + one standard error of the mean (n = 3).**

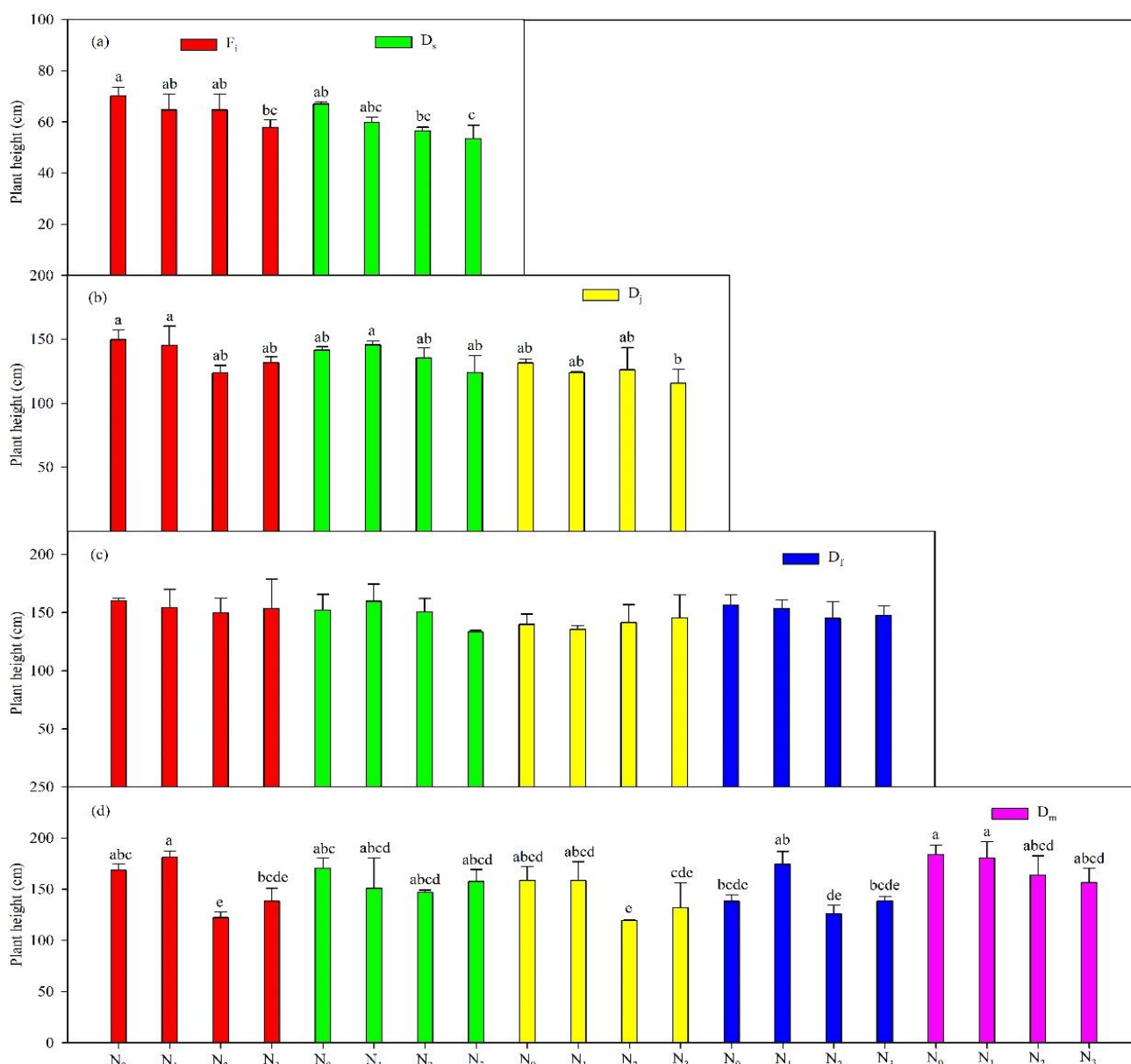


Fig. 2. Plant height per plant were affected by irrigation treatment (F<sub>i</sub>, full irrigation in the whole growth stage; D<sub>s</sub>, deficit irrigation was only applied in the seedling stage; D<sub>j</sub>, deficit irrigation was only applied in the jointing stage; D<sub>i</sub>, deficit irrigation was only applied in the maturity stage) and N-fertilizer application rates (N<sub>0</sub>, no N-fertilizer applied; N<sub>1</sub>, N-fertilizer applied as 0.1 g N kg<sup>-1</sup> soil; N<sub>2</sub>, N-fertilizer applied as 0.3 g N kg<sup>-1</sup> soil; N<sub>3</sub>, N-fertilizer applied as 0.5 g N kg<sup>-1</sup> soil) in seedling stage (a), jointing stage (b), filling stage (c) and maturity stage (d). Bars are the means + one standard error of the mean (n = 3).

At filling stage, there was no significant difference between the deficit irrigation and N-fertilizer application rate on plant height (Fig. 2c). On averaging N-fertilizer application rates, the order of plant height from high to low are F<sub>i</sub> (154 cm) > D<sub>i</sub> (151 cm) > D<sub>s</sub> (149 cm) > D<sub>j</sub> (141 cm) at filling stage (Fig. 2c). At maturity stage, the mean plant height in D<sub>m</sub> (171 cm) was 12.1% (19 cm), 9.4% (15 cm), 20.5% (29 cm) and 18.7% (27 cm) higher than F<sub>i</sub>, D<sub>s</sub>, D<sub>j</sub> and D<sub>m</sub> on averaging N-fertilizer application rates, respectively (Fig. 2d). On averaging irrigation treatments, the mean plant height was also decreased with the increase of N-fertilizer application rate, the mean plant height was higher in N<sub>1</sub> (211 cm) treatment, which was 24.6% (33 cm) and 16.9% (24 cm) higher than N<sub>2</sub> and N<sub>3</sub> at maturity stage (Fig. 2d).

**Chlorophyll content:** The N-fertilizer significantly affected the chlorophyll content in the whole growth stage, there was no significant difference in the single factor of irrigation and the interactions between irrigation and N-fertilizer on chlorophyll content during the whole growth stage (Table 1). At seedling stage, the highest chlorophyll content value was observed in N<sub>1</sub> (47.1) with F<sub>i</sub>, which was only significantly higher than N<sub>0</sub> (42.8) at the same irrigation treatment (Fig. 3a). At jointing stage, the mean chlorophyll content was increased with the increase of N-fertilizer application rate, and the value in N<sub>3</sub> (46) was 1.7% (0.8), 4.9% (2.1) and 16.9% (6.6) higher than N<sub>2</sub>, N<sub>1</sub> and N<sub>0</sub> on averaging irrigation treatments, respectively (Fig. 3b). On averaging N-fertilizer application rates, the order of chlorophyll content from high to low are D<sub>s</sub> (43.7) > D<sub>j</sub> (42.9) > F<sub>i</sub> (42.8) at jointing stage (Fig. 3b).

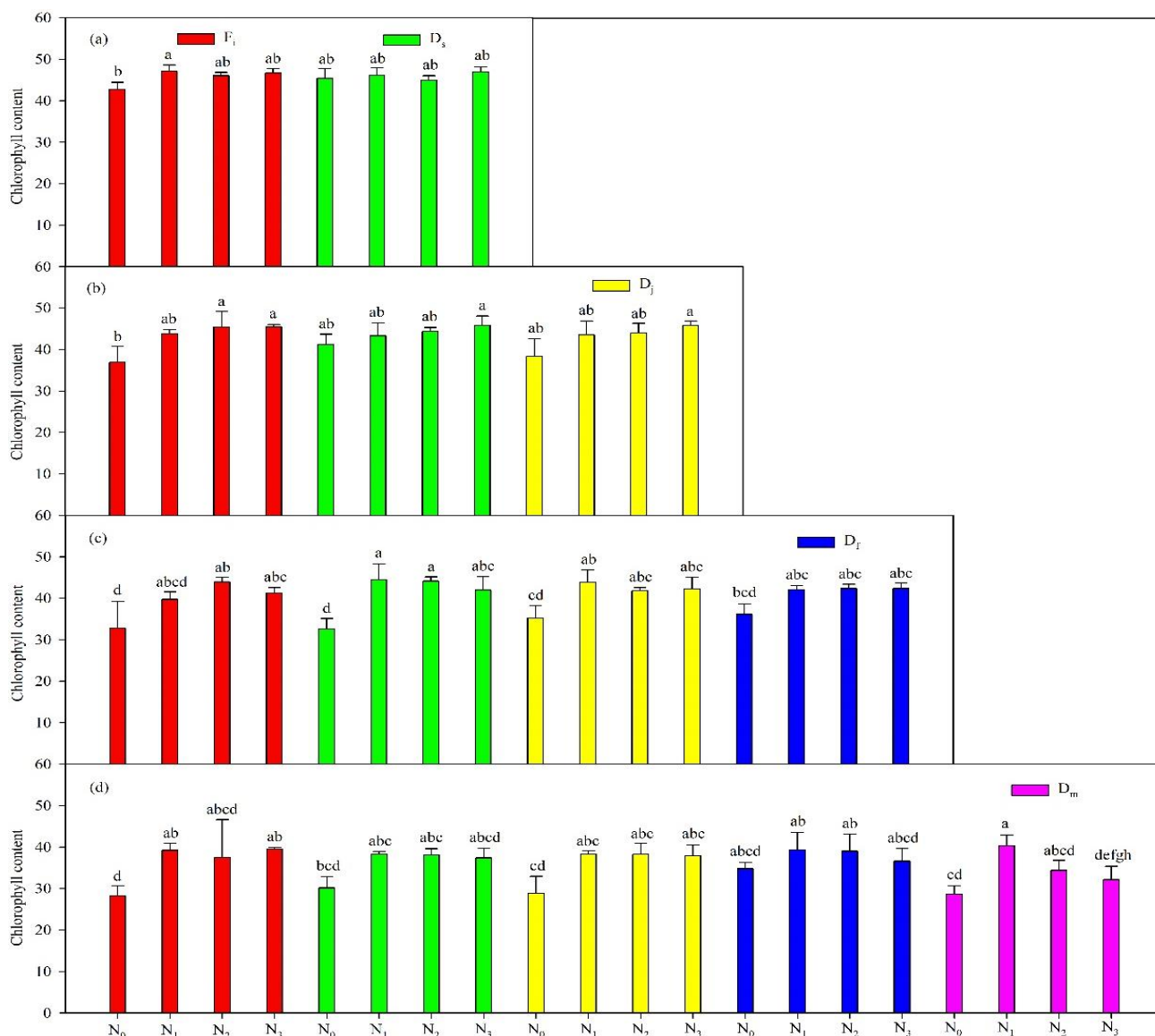


Fig. 3. Chlorophyll content (SPAD value) were affected by irrigation treatment (F<sub>1</sub>, full irrigation in the whole growth stage; D<sub>s</sub>, deficit irrigation was only applied in the seedling stage; D<sub>j</sub>, deficit irrigation was only applied in the jointing stage; D<sub>f</sub>, deficit irrigation was only applied in the filling stage; D<sub>m</sub>, deficit irrigation was only applied in the maturity stage) and N-fertilizer application rates (N<sub>0</sub>, no N-fertilizer applied; N<sub>1</sub>, N-fertilizer applied as 0.1 g N kg<sup>-1</sup> soil; N<sub>2</sub>, N-fertilizer applied as 0.3 g N kg<sup>-1</sup> soil; N<sub>3</sub>, N-fertilizer applied as 0.5 g N kg<sup>-1</sup> soil) in seedling stage (a), jointing stage (b), filling stage (c) and maturity stage (d). Bars are the means + one standard error of the mean ( $n = 3$ ).

At filling stage, the chlorophyll content was increased first and then decreased with the increase of N-fertilizer application rates (Fig. 3c). On averaging irrigation treatments, the order of chlorophyll content from high to low are N<sub>2</sub> (43.3) > N<sub>1</sub> (42.7) > N<sub>3</sub> (41.8) > N<sub>0</sub> (33.5) at filling stage (Fig. 3c). At maturity stage, the mean chlorophyll content in D<sub>f</sub> (37.4) was 3.6% (1.3), 4% (1.5), 4.4% (1.6) and 9.4% (3.5) higher than F<sub>1</sub>, D<sub>s</sub>, D<sub>j</sub> and D<sub>m</sub> on averaging N-fertilizer application rates, respectively (Fig. 3d). On averaging irrigation treatments, the mean chlorophyll content was decreased with the increase of N-fertilizer application rate, the mean chlorophyll content was higher in N<sub>1</sub> (39.1), which was 4.4% (1.6) and 6.4% (2.4) higher than N<sub>2</sub> and N<sub>3</sub> at maturity stage (Fig. 3d).

**Root number:** The individual factor of irrigation and N-fertilizer significantly affected the root number in the

whole growth stage except the irrigation effect on root number at seedling stage, however, there was no significant difference in the interactions between irrigation and N-fertilizer on root number during the whole growth stage (Table 1). The ability of root hyperplasia depends on the amount of N-fertilizer applied and the trend of root number from high to low are N<sub>2</sub> > N<sub>1</sub> > N<sub>3</sub> > N<sub>0</sub> at jointing stage (Fig. 4). In the whole growth stage, seedling and jointing stage are the periods of rapid growth of the root number, and the growth ability of root system is weakened during filling and maturity stage (Fig. 4). At seedling stage, the mean root number was increased first and then decreased with the increase of N-fertilizer application rates on averaging irrigation treatments (Fig. 4a). Compared with F<sub>1</sub>, the root number in D<sub>j</sub> reduce by 26% in N<sub>0</sub>, 25.8% in N<sub>1</sub>, 25% in N<sub>2</sub> and 27.87% in N<sub>3</sub> at jointing stage, respectively (Fig. 4b).

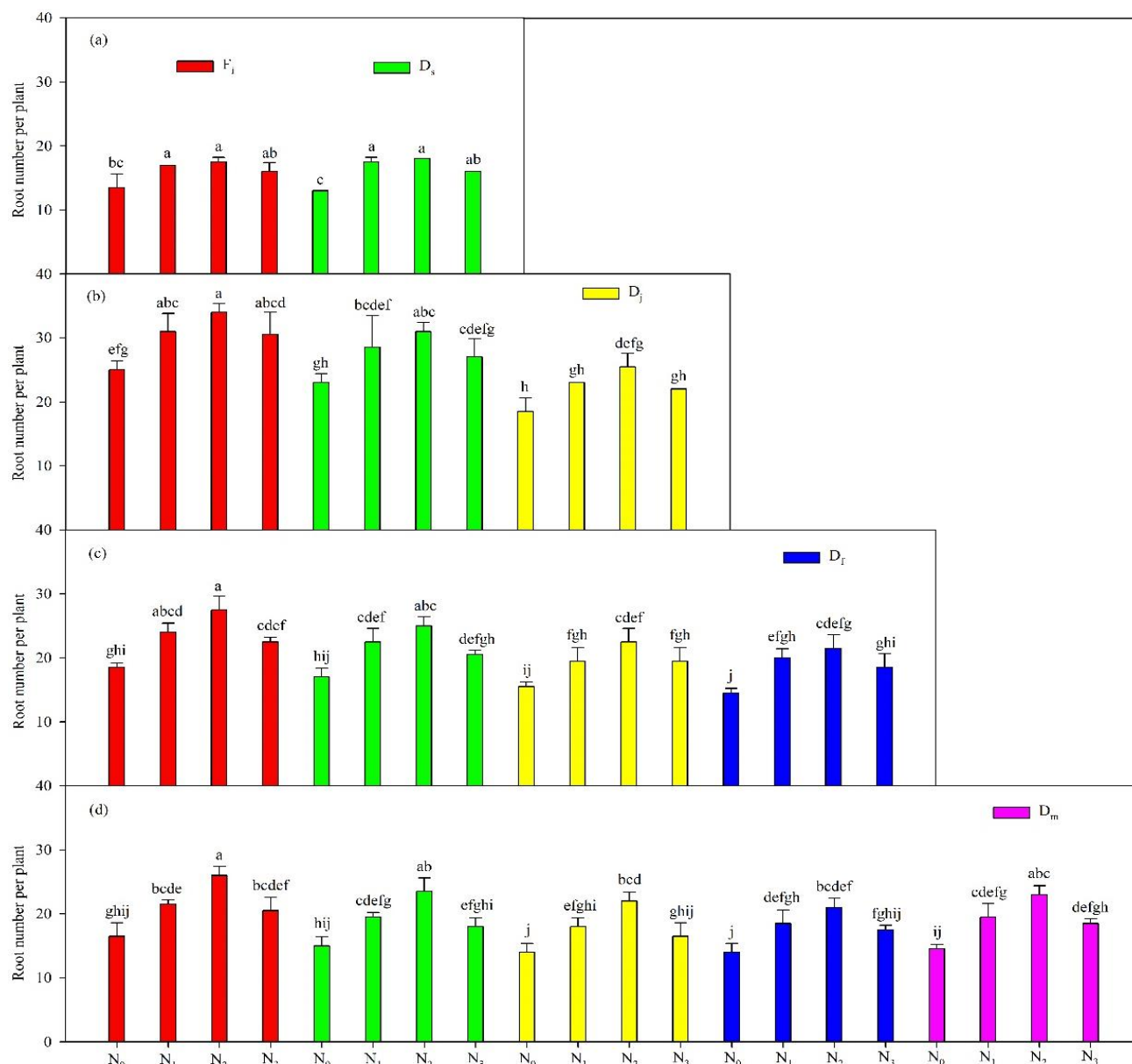


Fig. 4. Root number per plant were affected by irrigation treatment (F<sub>i</sub>, full irrigation in the whole growth stage; D<sub>s</sub>, deficit irrigation was only applied in the seedling stage; D<sub>j</sub>, deficit irrigation was only applied in the jointing stage; D<sub>r</sub>, deficit irrigation was only applied in the filling stage; D<sub>m</sub>, deficit irrigation was only applied in the maturity stage) and N-fertilizer application rates (N<sub>0</sub>, no N-fertilizer applied; N<sub>1</sub>, N-fertilizer applied as 0.1 g N kg<sup>-1</sup> soil; N<sub>2</sub>, N-fertilizer applied as 0.3 g N kg<sup>-1</sup> soil; N<sub>3</sub>, N-fertilizer applied as 0.5 g N kg<sup>-1</sup> soil) in seedling stage (a), jointing stage (b), filling stage (c) and maturity stage (d). Bars are the means + one standard error of the mean ( $n = 3$ ).

At filling stage, the root number in D<sub>j</sub> have a clear recovery, and the root number in D<sub>j</sub> reduce by 16.2% in N<sub>0</sub>, 18.8% in N<sub>1</sub>, 18.2% in N<sub>2</sub> and 13.3% in N<sub>3</sub> compare with F<sub>i</sub> at the same N-fertilizer application rate, respectively (Fig. 4c). However, the recovery ability of full irrigation at later of jointing stage is limited. The root number in D<sub>j</sub> reduce by 15.2% in N<sub>0</sub>, 16.3% in N<sub>1</sub>, 15.4% in N<sub>2</sub> and 19.5% in N<sub>3</sub> compare with F<sub>i</sub> under the same N-fertilizer application rate at maturity stage, respectively (Fig. 4d). In particular, deficit irrigation at maturity stage (D<sub>m</sub>) was also decreased the root number, which reduce by 12.1% in N<sub>0</sub>, 9.3% in N<sub>1</sub>, 11.5% in N<sub>2</sub> and 9.8% in N<sub>3</sub> compare with F<sub>i</sub> under the same N-fertilizer application rate at maturity stage, respectively (Fig. 4d).

**Root length:** The N-fertilizer significantly affected the root length in the whole growth stage, but there was no significant difference in the single factor of irrigation and the interactions between irrigation and N-fertilizer on root length during the whole growth stage (Table 1). At seedling stage, the root length was decreased with the increase of N-fertilizer application rates, and the root length in N<sub>1</sub> (38 cm) was 17.1% and 48.8% higher than N<sub>2</sub> (38 cm) and N<sub>3</sub> (30 cm) on averaging irrigation treatments (Fig. 5a). At jointing stage, the mean root length was increased first and then decreased with the increase of N-fertilizer application rate, and the value in N<sub>2</sub> (59 cm) was 17.1% (9 cm) and 65.3% (23 cm) higher than N<sub>1</sub> and N<sub>3</sub> on averaging irrigation treatments, respectively (Fig. 5b). On averaging N-fertilizer application rates, the order of root length from high to low are F<sub>i</sub> (53.1) > D<sub>s</sub> (51.6) > D<sub>j</sub> (50.6) at jointing stage (Fig. 5b).



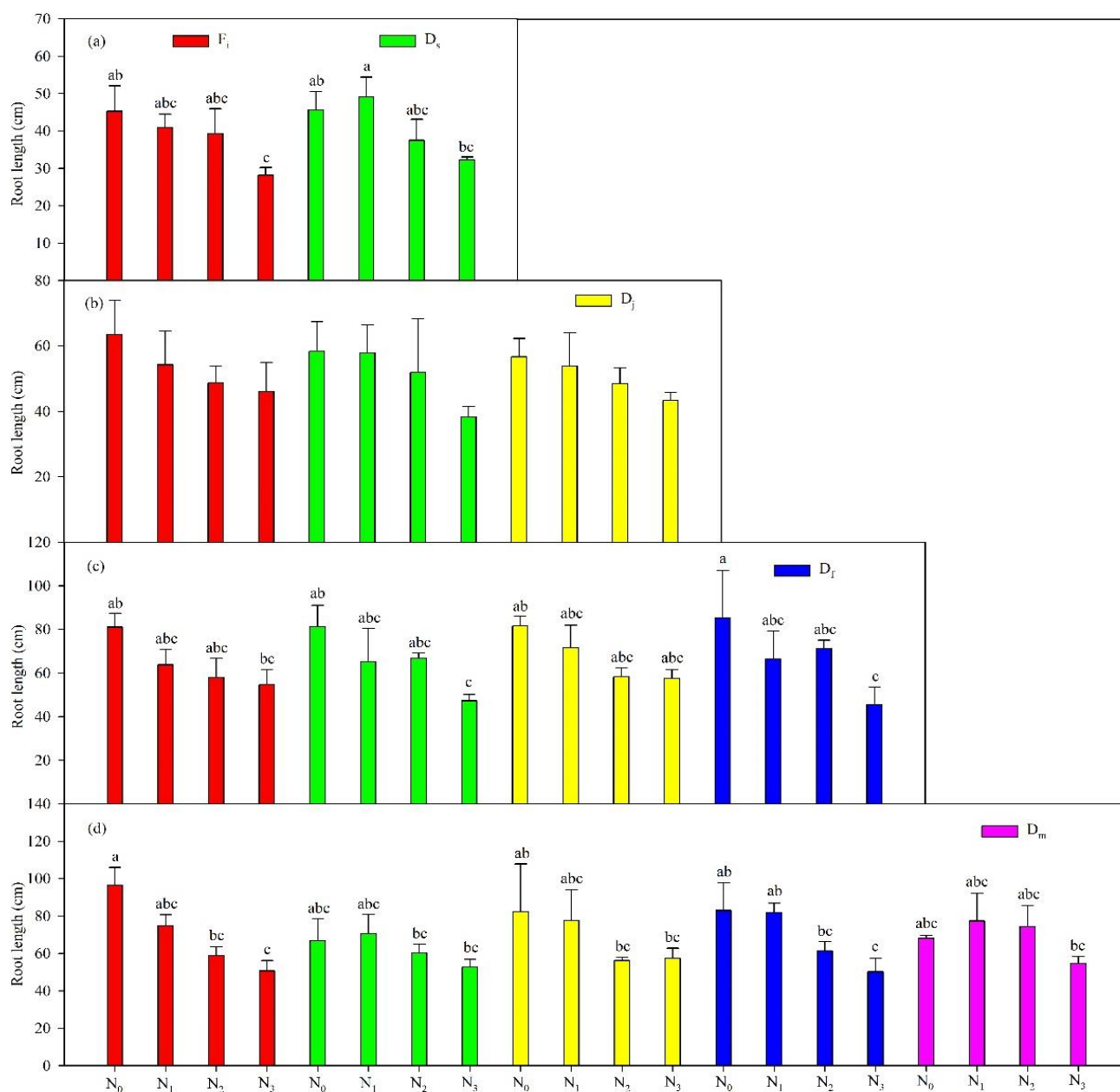


Fig. 5. Root length were affected by irrigation treatment ( $F_1$ , full irrigation in the whole growth stage;  $D_s$ , deficit irrigation was only applied in the seedling stage;  $D_j$ , deficit irrigation was only applied in the jointing stage;  $D_f$ , deficit irrigation was only applied in the filling stage;  $D_m$ , deficit irrigation was only applied in the maturity stage) and N-fertilizer application rates ( $N_0$ , no N-fertilizer applied;  $N_1$ , N-fertilizer applied as  $0.1 \text{ g N kg}^{-1}$  soil;  $N_2$ , N-fertilizer applied as  $0.3 \text{ g N kg}^{-1}$  soil;  $N_3$ , N-fertilizer applied as  $0.5 \text{ g N kg}^{-1}$  soil) in seedling stage (a), jointing stage (b), filling stage (c) and maturity stage (d). Bars are the means + one standard error of the mean ( $n = 3$ ).

At filling stage, the positive relationship between root length and deficit irrigation, and the order of root length from high to low are  $D_j > D_f > D_s > F_1$  on averaging N-fertilizer application rate (Fig. 5c). On averaging irrigation treatments, the root length was decreased with the increase of N-fertilizer application rate, and the root length in  $N_1$  (65.2 cm) was 4.9% (3.1 cm) and 30.3% (15.5 cm) higher than  $N_2$  and  $N_3$  at filling stage, respectively (Fig. 5c). On averaging irrigation treatments, the mean root length was also decreased with the increase of N-fertilizer application rate, the mean root length was higher in  $N_1$  (76.5 cm), which was 22.8% (14.2 cm) and 44% (23.4 cm) higher than  $N_2$  and  $N_3$  at maturity stage, respectively (Fig. 5d).

**Root activity:** The individual factor of irrigation and N-fertilizer significantly ( $p < 0.01$ ) affected the root activity in the whole growth stage, and the interactions between irrigation and N-fertilizer have a significant effect on root activity at maturity stage (Table 1). The root activity decreased with the increase of N-fertilizer application rate, and the root activity order from high to low was  $N_1 > N_2 > N_3$  (Fig. 6a). On averaging N-fertilizer application rate, the root activity in  $D_s$  increased by 9.2%, compared with  $F_1$  at seedling stage (Fig. 6a). The root activity decreased with deficit irrigation, and the root activity order from high to low was  $D_s > F_1 > D_j$  on averaging N-fertilizer application rate at jointing stage (Fig. 6b). On averaging N-fertilizer application rate, the mean root activity in  $D_j$  decreased by 33.8% and 37%, compared with  $D_s$  and  $F_1$  at jointing stage (Fig. 6b).



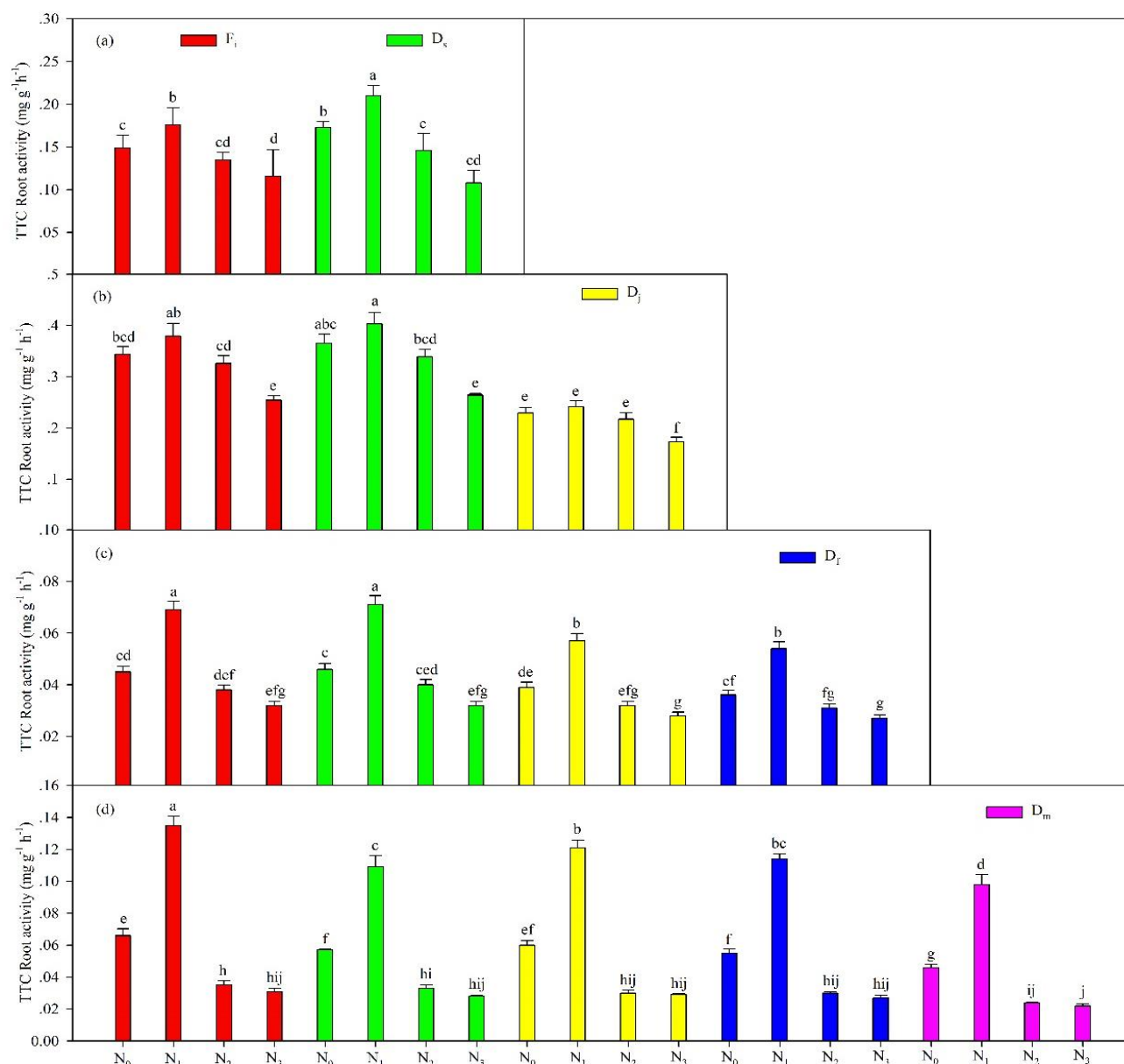


Fig. 6. Root activity were affected by irrigation treatment (F<sub>i</sub>, full irrigation in the whole growth stage; D<sub>s</sub>, deficit irrigation was only applied in the seedling stage; D<sub>j</sub>, deficit irrigation was only applied in the jointing stage; D<sub>r</sub>, deficit irrigation was only applied in the filling stage; D<sub>m</sub>, deficit irrigation was only applied in the maturity stage) and N-fertilizer application rates (N<sub>0</sub>, no N-fertilizer applied; N<sub>1</sub>, N-fertilizer applied as 0.1 g N kg<sup>-1</sup> soil; N<sub>2</sub>, N-fertilizer applied as 0.3 g N kg<sup>-1</sup> soil; N<sub>3</sub>, N-fertilizer applied as 0.5 g N kg<sup>-1</sup> soil) in seedling stage (a), jointing stage (b), filling stage (c) and maturity stage (d). Bars are the means + one standard error of the mean (n = 3).

Compared with F<sub>i</sub>, the root activity in D<sub>j</sub> reduce by 33.6% in N<sub>0</sub>, 36.4% in N<sub>1</sub>, 33.3% in N<sub>2</sub> and 31.7% in N<sub>3</sub> at filling stage (Fig. 6c). However, the root activity in D<sub>s</sub> reduce by 14.3% in N<sub>0</sub>, 19.5% in N<sub>1</sub>, 5% in N<sub>2</sub> and 7.9% in N<sub>3</sub>, compared with F<sub>i</sub> at filling stage (Fig. 6c). Compared with F<sub>i</sub>, the root activity in D<sub>m</sub> reduce by 30.4% in N<sub>0</sub>, 27.7% in N<sub>1</sub>, 31.7% in N<sub>2</sub> and 29.9% in N<sub>3</sub> at maturity stage (Fig. 6d).

**Dry matter accumulation:** The N-fertilizer significantly affected the dry matter accumulation in the whole growth stage and the irrigation was significant effect on dry matter accumulation at jointing stage, but there was no significant difference in the interactions between irrigation and N-fertilizer on dry matter accumulation (Table 1). At seedling stage, the dry matter accumulation

was decreased with the increase of N-fertilizer application rates, and the mean dry matter accumulation in N<sub>1</sub> (2.5 g plant<sup>-1</sup>) was 36.5% and 79.5% higher than N<sub>2</sub> and N<sub>3</sub> on averaging irrigation treatments, respectively (Fig. 7a). Compared with F<sub>i</sub>, the mean dry matter accumulation in D<sub>s</sub> reduced by 24.2% on averaging N-fertilizer application rate at seedling stage (Fig. 7a). At jointing stage, the dry matter accumulation was decreased with the increase of N-fertilizer application rates, and the mean dry matter accumulation in N<sub>1</sub> (36.4 g plant<sup>-1</sup>) was 24.3% and 61.8% higher than N<sub>2</sub> and N<sub>3</sub> on averaging irrigation treatments, respectively (Fig. 7b). On averaging N-fertilizer application rates, the deficit irrigation decreased the dry matter accumulation, and the mean value in F<sub>i</sub> increased by 33% than D<sub>s</sub> and increased by 35% than D<sub>j</sub> at jointing stage (Fig. 7b).

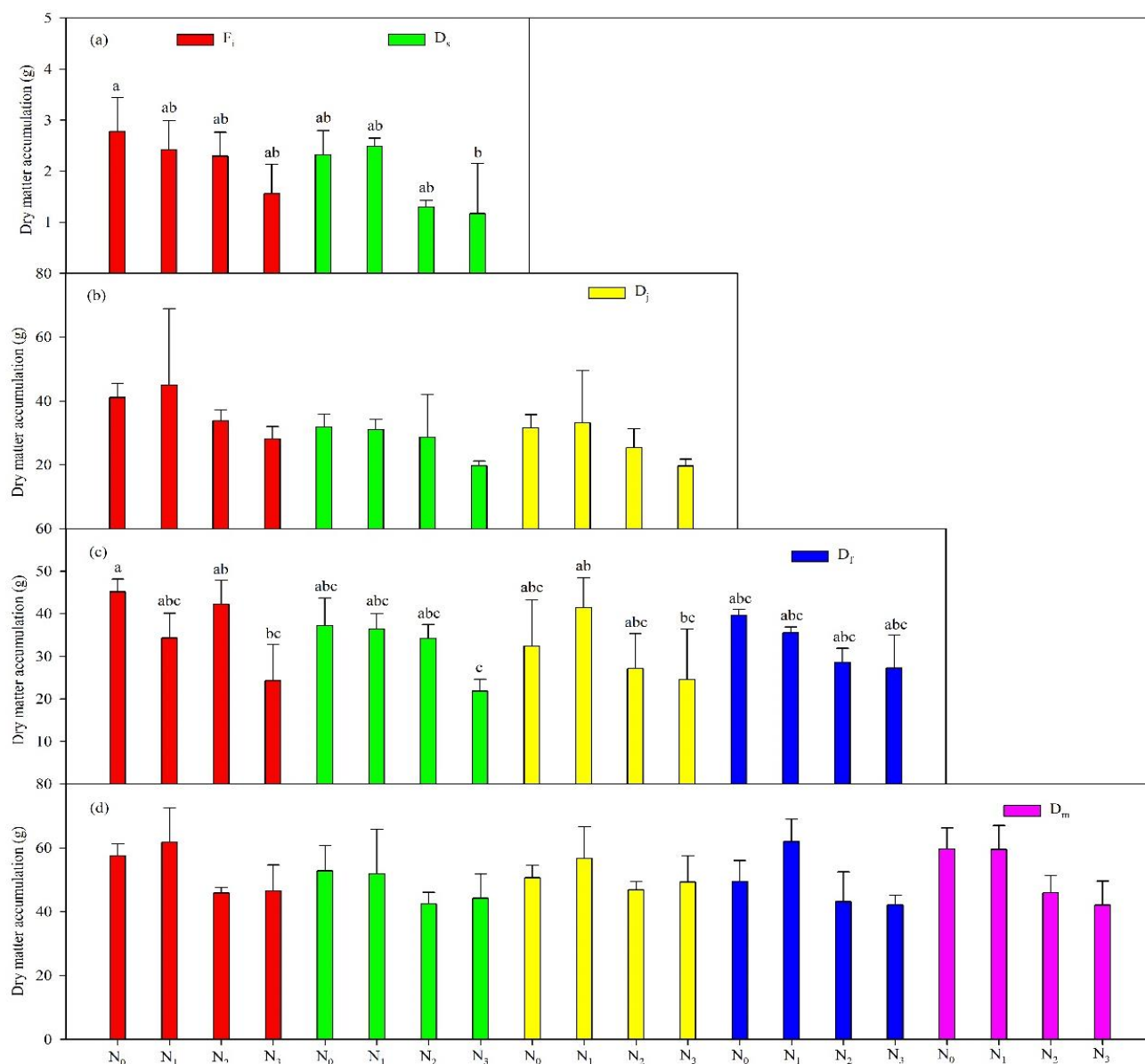


Fig. 7. Dry matter accumulation were affected by irrigation treatment (F<sub>i</sub>, full irrigation in the whole growth stage; D<sub>s</sub>, deficit irrigation was only applied in the seedling stage; D<sub>j</sub>, deficit irrigation was only applied in the jointing stage; D<sub>f</sub>, deficit irrigation was only applied in the filling stage; D<sub>m</sub>, deficit irrigation was only applied in the maturity stage) and N-fertilizer application rates (N<sub>0</sub>, no N-fertilizer applied; N<sub>1</sub>, N-fertilizer applied as 0.1 g N kg<sup>-1</sup> soil; N<sub>2</sub>, N-fertilizer applied as 0.3 g N kg<sup>-1</sup> soil; N<sub>3</sub>, N-fertilizer applied as 0.5 g N kg<sup>-1</sup> soil) in seedling stage (a), jointing stage (b), filling stage (c) and maturity stage (d). Bars are the means + one standard error of the mean ( $n = 3$ ).

At filling stage, the positive relationship between dry matter accumulation and deficit irrigation, and the highest dry matter accumulation was observed in F<sub>i</sub>, which was 12.5%, 16.3% and 11.3% higher than D<sub>s</sub>, D<sub>j</sub> and D<sub>f</sub> on averaging N-fertilizer application rate (Fig. 7c). On averaging irrigation treatments, the dry matter accumulation was decreased with the increase of N-fertilizer application rate, and the dry matter accumulation in N<sub>1</sub> (36.9 g plant<sup>-1</sup>) was 11.8% and 50.8% higher than N<sub>2</sub> and N<sub>3</sub> at filling stage, respectively (Fig. 7c). On averaging irrigation treatments, the mean dry matter accumulation was also decreased with the increase of N-fertilizer application rate, the mean dry matter accumulation was higher in N<sub>1</sub> (58.4 g plant<sup>-1</sup>), which was 30.1% and 30% higher than N<sub>2</sub> and N<sub>3</sub> at maturity stage, respectively (Fig. 7d). The dry matter accumulation decreased with deficit

irrigation, and the mean dry matter accumulation order from high to low was F<sub>i</sub> > D<sub>m</sub> > D<sub>j</sub> > D<sub>f</sub> > D<sub>s</sub> on averaging N-fertilizer application rate at maturity stage (Fig. 7d).

**Nitrogen uptake:** The individual irrigation and N-fertilizer significantly ( $p < 0.01$ ) affected the nitrogen uptake in the whole growth stage, and there have significant ( $p < 0.01$ ) interaction impact between them on nitrogen uptake (Table 1). High nitrogen application can significantly inhibit nitrogen uptake at seedling stage, the mean nitrogen uptake in N<sub>1</sub> (7.5 mg plant<sup>-1</sup>) was 16% and 103.3% higher than N<sub>2</sub> and N<sub>3</sub> on averaging irrigation treatments, respectively (Fig. 8a). however, the nitrogen uptake increased with irrigation amount, and the mean nitrogen uptake in F<sub>i</sub> increased by 19% compared with D<sub>s</sub> at seedling stage (Fig. 8a). At jointing stage, the high nitrogen continuous

inhibition of plant nitrogen uptake, the mean nitrogen uptake in  $N_1$  ( $77 \text{ mg plant}^{-1}$ ) was 21.7% and 45.1% higher than  $N_2$  and  $N_3$  on averaging irrigation treatments, respectively (Fig. 8b). Regularly, the mean nitrogen uptake order from high to low was  $F_i > D_s > D_j$  on averaging N-fertilizer application rate at jointing stage (Fig. 8b).

At filling stage, the nitrogen uptake still follows the rule that nitrogen uptake decreased with the increase of N-fertilizer application rate, and the mean value in  $N_1$  ( $72.2 \text{ mg plant}^{-1}$ ) was 1% and 23% higher than  $N_2$  and  $N_3$  on averaging irrigation treatments, respectively (Fig. 8c). Interestingly, the nitrogen uptake was not following the

positive relationship with irrigation, and the nitrogen uptake order from high to low was  $D_s > F_i > D_f > D_j$  on averaging N-fertilizer application rate at filling stage (Fig. 8c). At maturity stage, N-fertilizer can significantly increase nitrogen uptake and the highest value of nitrogen uptake was observed in  $N_1$ , which was 105.3% ( $39.6 \text{ mg plant}^{-1}$ ) higher than  $N_0$  on averaging irrigation treatments (Fig. 8d). Deficit irrigation at jointing stage is the most influential stage of nitrogen uptake, compared the nitrogen uptake in  $D_j$ , which increased nitrogen uptake by 20.7% in  $F_i$ , 12.7% in  $D_s$ , 7.6% in  $D_f$  and 14.1% in  $D_m$  on averaging N-fertilizer application rate at maturity stage (Fig. 8d).

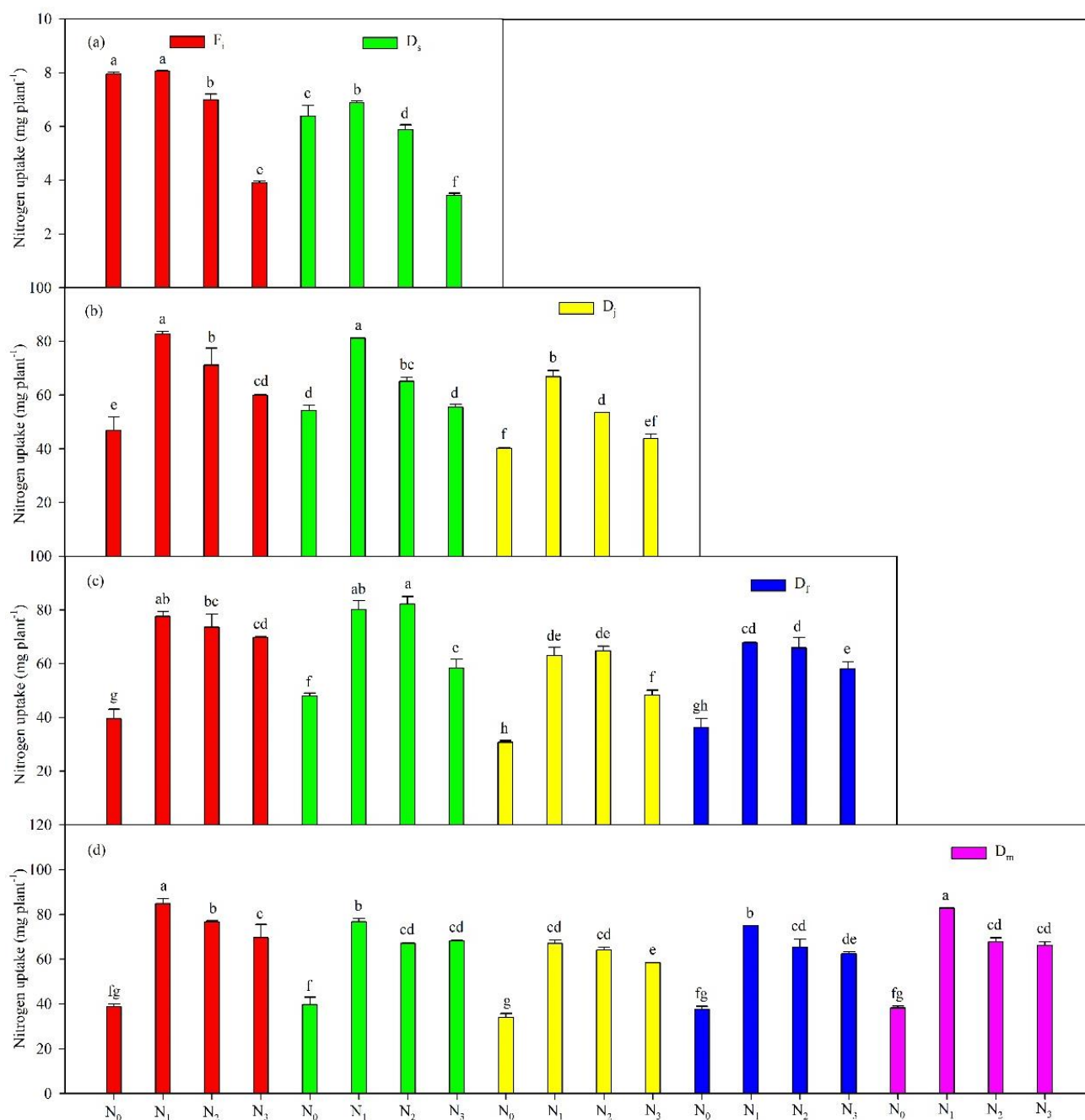


Fig. 8. Nitrogen uptake per plant were affected by irrigation treatment ( $F_i$ , full irrigation in the whole growth stage;  $D_s$ , deficit irrigation was only applied in the seedling stage;  $D_j$ , deficit irrigation was only applied in the jointing stage;  $D_f$ , deficit irrigation was only applied in the filling stage;  $D_m$ , deficit irrigation was only applied in the maturity stage) and N-fertilizer application rates ( $N_0$ , no N-fertilizer applied;  $N_1$ , N-fertilizer applied as  $0.1 \text{ g N kg}^{-1}$  soil;  $N_2$ , N-fertilizer applied as  $0.3 \text{ g N kg}^{-1}$  soil;  $N_3$ , N-fertilizer applied as  $0.5 \text{ g N kg}^{-1}$  soil) in seedling stage (a), jointing stage (b), filling stage (c) and maturity stage (d). Bars are the means + one standard error of the mean ( $n = 3$ ).

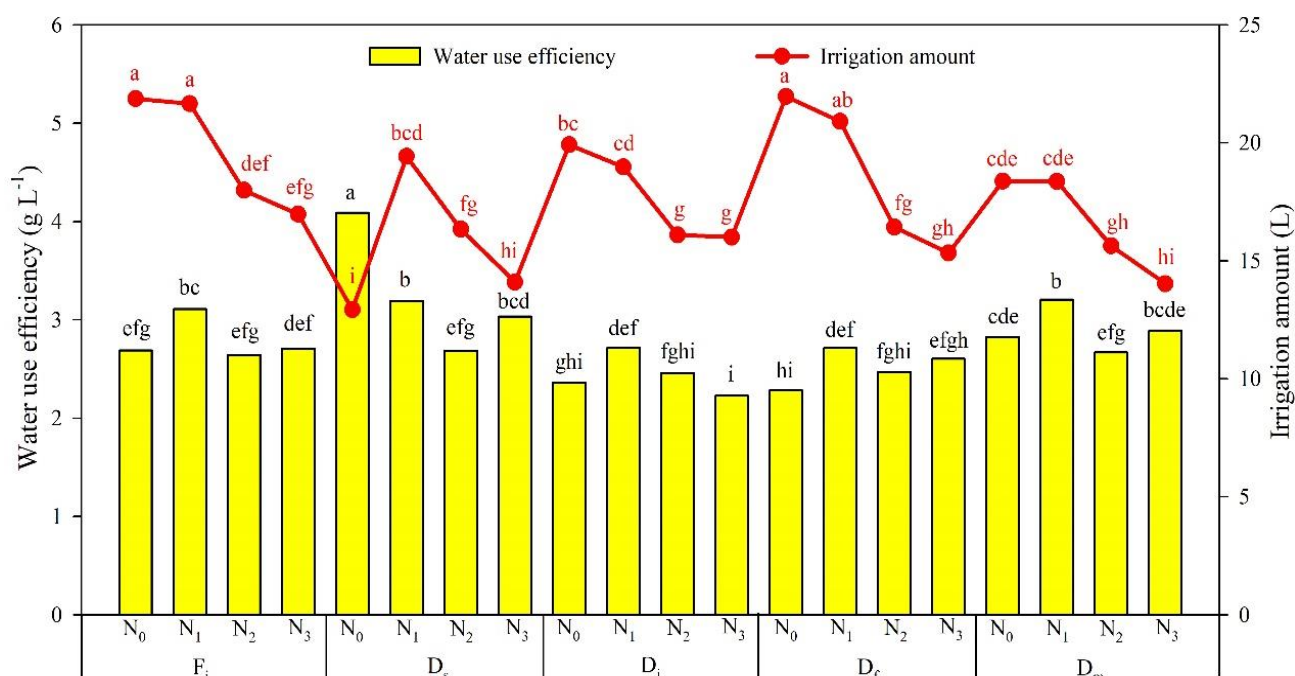


Fig. 9. Water use efficiency and irrigation amount were affected by irrigation treatment (F<sub>i</sub>, full irrigation in the whole growth stage; D<sub>s</sub>, deficit irrigation was only applied in the seedling stage; D<sub>j</sub>, deficit irrigation was only applied in the jointing stage; D<sub>r</sub>, deficit irrigation was only applied in the filling stage; D<sub>m</sub>, deficit irrigation was only applied in the maturity stage) and N-fertilizer application rates (N<sub>0</sub>, no N-fertilizer applied; N<sub>1</sub>, N-fertilizer applied as 0.1 g N kg<sup>-1</sup> soil; N<sub>2</sub>, N-fertilizer applied as 0.3 g N kg<sup>-1</sup> soil; N<sub>3</sub>, N-fertilizer applied as 0.5 g N kg<sup>-1</sup> soil) in maturity stage.

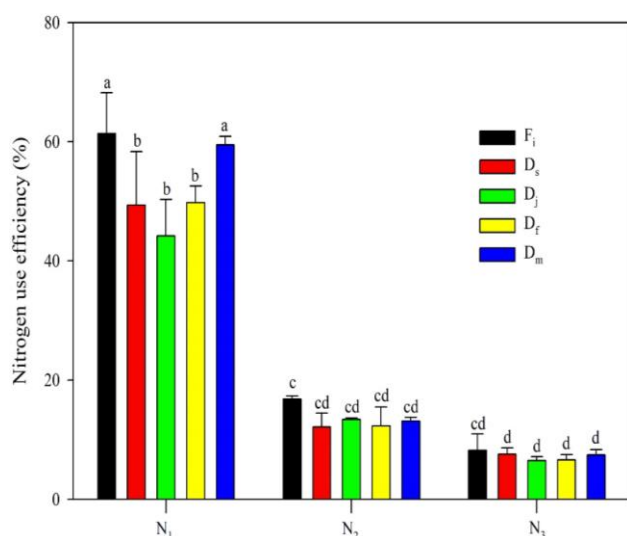


Fig. 10. Nitrogen use efficiency were affected by irrigation treatment (F<sub>i</sub>, full irrigation in the whole growth stage; D<sub>s</sub>, deficit irrigation was only applied in the seedling stage; D<sub>j</sub>, deficit irrigation was only applied in the jointing stage; D<sub>r</sub>, deficit irrigation was only applied in the filling stage; D<sub>m</sub>, deficit irrigation was only applied in the maturity stage) and N-fertilizer application rates (N<sub>1</sub>, N-fertilizer applied as 0.1 g N kg<sup>-1</sup> soil; N<sub>2</sub>, N-fertilizer applied as 0.3 g N kg<sup>-1</sup> soil; N<sub>3</sub>, N-fertilizer applied as 0.5 g N kg<sup>-1</sup> soil) in maturity stage.

**Water use efficiency and nitrogen use efficiency:** The negative correlation between irrigation amount and water use efficiency (WUE), and the highest WUE (4.1 g L<sup>-1</sup>) corresponding to the lowest irrigation amount (12.9 L) that obtained in F<sub>i</sub> with N<sub>0</sub> (Fig. 9). On averaging N-fertilizer application rate, the mean WUE

order from high to low was D<sub>s</sub> > D<sub>m</sub> > F<sub>i</sub> > D<sub>r</sub> > D<sub>j</sub> (Fig. 9). The mean WUE in N<sub>1</sub> was 4.8%, 15.6% and 10.95 higher than N<sub>0</sub>, N<sub>2</sub> and N<sub>3</sub>, respectively (Fig. 9). Surprisingly, the WUE was only significant correlated with plant height (Table 2).

There has a negative correlation between N-fertilizer application rate and nitrogen use efficiency (NUE), and the mean NUE in N<sub>1</sub> (54.4%) was 73.4% and 85.8% higher than N<sub>2</sub> and N<sub>3</sub>, respectively (Fig. 10). The positive relationship between irrigation amount and NUE, and the mean NUE order from high to low was F<sub>i</sub> (31.2%) > D<sub>m</sub> (26.3%) > D<sub>s</sub> (25.9%) > F<sub>r</sub> (24.3%) > D<sub>j</sub> (19.7%) (Fig. 10). The NUE was significant correlated with leaf area, chlorophyll content, root activity, dry matter accumulation and nitrogen uptake (Table 2).

**Rankings of all the parameters based on a PCA:** The means of the leaf area, plant height, chlorophyll content, root number, root length, root activity, dry matter accumulation, nitrogen uptake, water use efficiency and nitrogen use efficiency at maturity stage were collected in Table 3. This value was then used in the following steps: (1) the data were converted to standardized values (Table 4), (2) the correlation matrix was calculated using the standardized values, and (3) the total variance explained of the contribution rate and accumulative of contribution rate with eigenvalues, was obtained via a PCA. In this analysis, three components were extracted from the matrix of all the parameters. Finally, the score and rank of the comprehensive all the parameters calculated in a PCA (Table 5); The top five are F<sub>i</sub>N<sub>1</sub>, D<sub>m</sub>N<sub>1</sub>, D<sub>s</sub>N<sub>1</sub>, D<sub>r</sub>N<sub>1</sub> and D<sub>j</sub>N<sub>1</sub>, undoubtedly, the D<sub>j</sub>N<sub>0</sub> was clearly last.

**Table 2. The correlation among the leaf area (LA), plant height (PH), chlorophyll content (CC), root number (RN), root length (RL), root activity (RA), dry matter accumulation (DMA), nitrogen uptake (NU), water use efficiency (WUE) and nitrogen use efficiency (NUE) under irrigation and N-fertilizer application rate.**

Items	LA	PH	CC	RN	RL	RA	DMA	NU	WUE	NUE
LA	1.000	-0.038	0.441	0.334	-0.058	0.464*	0.193	0.474*	0.062	0.534*
PH		1.000	-0.341	-0.348	0.541*	0.558*	0.726**	-0.061	0.544*	0.360
CC			1.000	0.593**	-0.355	0.222	-0.093	0.841**	-0.113	0.623**
RN				1.000	-0.342	-0.100	-0.311	0.752**	-0.056	0.335
RL					1.000	0.614**	0.697**	-0.315	0.002	0.258
RA						1.000	0.818**	0.293	0.315	0.830**
DMA							1.000	-0.018	0.287	0.560*
NU								1.000	0.124	0.751**
WUE									1.000	0.265
NUE										1.000

Note: \*\*\* means  $p < 0.01$ , \*\* means  $0.01 < p < 0.05$

**Table 3. Mean values of leaf area (LA, cm<sup>2</sup>), plant height (PH, cm), chlorophyll content (CC), root number (RN), root length (RL, cm), root activity (RA, mg g<sup>-1</sup> h<sup>-1</sup>), dry matter accumulation (DMA, g plant<sup>-1</sup>), nitrogen uptake (NU, mg plant<sup>-1</sup>), water use efficiency (WUE, g L<sup>-1</sup>) and nitrogen use efficiency (NUE, %) at maturity stage.**

Items	LA	PH	CC	RN	RL	RA	DMA	NU	WUE	NUE
FiN0	3201.78	168.60	28.20	16.50	96.52	0.07	57.54	38.72	2.69	0.00
FiN1	4652.28	181.30	39.20	21.50	74.95	0.14	61.75	84.78	3.11	61.41
FiN2	3734.79	122.30	37.47	26.00	58.92	0.04	45.88	76.61	2.64	16.84
FiN3	3421.41	138.43	39.57	20.50	50.73	0.03	46.57	69.61	2.71	8.24
DsN0	3803.27	170.60	30.17	15.00	66.87	0.06	52.79	39.74	4.09	0.00
DsN1	3864.34	151.00	38.23	19.50	70.55	0.11	51.88	76.79	3.19	49.40
DsN2	3664.13	146.87	38.10	23.50	60.42	0.03	42.43	67.11	2.69	12.17
DsN3	3129.30	157.57	37.40	18.00	52.72	0.03	44.25	68.10	3.03	7.56
DjN0	3263.68	158.50	28.83	14.00	82.48	0.06	50.64	33.92	2.36	0.00
DjN1	4818.94	158.53	38.33	18.00	77.63	0.12	56.71	67.07	2.71	44.20
DjN2	3913.97	119.37	38.27	22.00	56.23	0.03	46.88	64.07	2.46	13.40
DjN3	3899.21	132.04	37.93	16.50	57.28	0.03	49.29	58.37	2.23	6.52
DfN0	2892.31	138.20	34.77	14.00	83.07	0.06	49.45	37.59	2.28	0.00
DfN1	4362.98	174.33	39.30	18.50	81.88	0.11	61.93	74.94	2.71	49.81
DfN2	4069.81	125.80	39.03	21.00	61.40	0.03	43.15	65.43	2.47	12.37
DfN3	3898.27	138.53	36.60	17.50	50.22	0.03	42.14	62.44	2.60	6.63
DmN0	3176.50	183.80	28.70	14.50	68.15	0.05	59.68	38.20	2.83	0.00
DmN1	3057.86	180.30	40.37	19.50	77.42	0.10	59.52	82.82	3.21	59.49
DmN2	3685.96	164.03	34.40	23.00	74.42	0.02	45.91	67.77	2.67	13.14
DmN3	3565.08	156.63	32.17	18.50	54.70	0.02	42.09	66.11	2.89	7.44

Notes: Fi, full irrigation in the whole growth stage; Ds, deficit irrigation was only applied in the seedling stage; Dj, deficit irrigation was only applied in the jointing stage; Df, deficit irrigation was only applied in the filling stage; Dm, deficit irrigation was only applied in the maturity stage; N0, no N-fertilizer applied; N1, N-fertilizer applied as 0.1 g N kg<sup>-1</sup> soil; N2, N-fertilizer applied as 0.3 g N kg<sup>-1</sup> soil; N3, N-fertilizer applied as 0.5 g N kg<sup>-1</sup> soil

**Table 4. The standardized values of all the indicators in irrigation and N-fertilizer treatments.**

Items	LA	PH	CC	RN	RL	RA	DMA	NU	WUE	NUE
FiN0	-0.97	0.76	-1.90	-0.71	2.22	0.23	1.02	-1.47	-0.21	-0.87
FiN1	1.83	1.39	0.83	0.79	0.55	2.09	1.64	1.44	0.80	2.02
FiN2	0.06	-1.55	0.40	2.14	-0.69	-0.61	-0.68	0.92	-0.33	-0.07
FiN3	-0.55	-0.74	0.92	0.49	-1.32	-0.72	-0.58	0.48	-0.16	-0.48
DsN0	0.19	0.86	-1.41	-1.16	-0.07	-0.01	0.33	-1.41	3.16	-0.87
DsN1	0.31	-0.12	0.59	0.19	0.21	1.39	0.20	0.93	0.99	1.46
DsN2	-0.08	-0.32	0.56	1.39	-0.57	-0.66	-1.18	0.32	-0.21	-0.29
DsN3	-1.11	0.21	0.38	-0.26	-1.17	-0.80	-0.92	0.38	0.61	-0.51
DjN0	-0.85	0.26	-1.74	-1.46	1.13	0.07	0.02	-1.77	-1.01	-0.87
DjN1	2.15	0.26	0.62	-0.26	0.76	1.71	0.90	0.32	-0.16	1.21
DjN2	0.41	-1.69	0.60	0.94	-0.90	-0.74	-0.53	0.13	-0.77	-0.24
DjN3	0.38	-1.06	0.52	-0.71	-0.81	-0.77	-0.18	-0.23	-1.32	-0.56
DfN0	-1.57	-0.75	-0.27	-1.46	1.18	-0.07	-0.16	-1.54	-1.20	-0.87
DfN1	1.27	1.05	0.86	-0.11	1.09	1.53	1.67	0.82	-0.16	1.48
DfN2	0.71	-1.37	0.79	0.64	-0.50	-0.74	-1.08	0.22	-0.74	-0.28
DfN3	0.38	-0.74	0.19	-0.41	-1.36	-0.82	-1.22	0.03	-0.43	-0.55
DmN0	-1.02	1.52	-1.78	-1.31	0.02	-0.31	1.34	-1.50	0.12	-0.87
DmN1	-1.25	1.34	1.12	0.19	0.74	1.09	1.31	1.31	1.04	1.93
DmN2	-0.03	0.53	-0.36	1.24	0.51	-0.90	-0.67	0.36	-0.26	-0.25
DmN3	-0.27	0.16	-0.92	-0.11	-1.01	-0.96	-1.23	0.26	0.27	-0.52

Note: The abbreviation stands for the same meaning as above

**Table 5. The score and rank of the comprehensive leaf area, plant height, chlorophyll content, root number, root length, root activity, dry matter accumulation, nitrogen uptake, water use efficiency and nitrogen use efficiency at maturity stage, which calculated in a PCA of the irrigation and N-fertilizer treatments.**

Items	F1	F2	F3	F	Rank
FiN0	-0.18	-1.89	-0.79	-0.80	18
FiN1	2.29	0.29	0.11	0.98	1
FiN2	-0.38	1.41	-0.06	0.33	6
FiN3	-0.59	0.89	0.42	0.13	8
DsN0	-0.07	-1.38	2.53	-0.21	15
DsN1	1.10	0.35	0.38	0.58	3
DsN2	-0.46	0.92	0.30	0.17	7
DsN3	-0.62	0.29	1.36	0.01	11
DjN0	-0.75	-1.56	-1.11	-0.94	20
DjN1	1.42	0.08	-1.11	0.44	5
DjN2	-0.61	1.10	-0.61	0.07	10
DjN3	-0.71	0.42	-1.05	-0.24	16
DfN0	-0.90	-1.01	-1.56	-0.86	19
DfN1	1.72	-0.12	-0.89	0.52	4
DfN2	-0.57	1.07	-0.65	0.07	9
DfN3	-0.84	0.63	0.16	-0.09	13
DmN0	-0.38	-1.76	0.51	-0.69	17
DmN1	1.54	-0.12	0.77	0.63	2
DmN2	-0.24	0.25	0.18	0.01	12
DmN3	-0.78	0.15	1.11	-0.12	14

Note: The abbreviation stands for the same meaning as above



## Discussion

Leaf area is an important factor for plant growth, which is related to the plants' competition ability (Wang *et al.*, 2015; Gu *et al.*, 2018). Other studies show that leaf area positively related to plant height and dry matter accumulation, which is linked to the relative growth rate (Santini *et al.*, 2017). However, there was no significant correlation relationship between leaf area and plant height in this study (Table 2). We show that the leaf area has been well correlated with root activity, nitrogen uptake and nitrogen use efficiency (Table 2). Deficit irrigation was only improving leaf area at seedling stage, and the leaf area growth was significantly suppressed by deficit irrigation during jointing and filling stage (Fig. 1). A similar result was also reported that water stress negative influence of the leaf expansion and rise in plant height (Kuang *et al.*, 2014; Zhang *et al.*, 2015). In this study, plant height was strongly associated with root length, root activity, dry matter accumulation and water use efficiency (Table 2). Plant height was slightly lower in low soil moisture content, and a similar result was found that plant height have a linear dependence relation with dry matter accumulation (Lipiec *et al.*, 1996). Our result indicated that plant height has a significant influence by individual factor of irrigation and N-fertilizer. We can conclude that plant height is a very sensitive indicator of maize growth, and it has four times over the significant level (Table 2). We can suggest that improving plant height is an effective measure for maize growth in field managers and maize plant breeders, which help to select genotypes with a plant height adapted to the conditions of the target environment.

Root number is a potential trait for nitrogen acquisition from low nitrogen soils (Saengwilai *et al.*, 2014), and the root number and length is important for maize productivity under soil water and nutrients stress (Magaia *et al.*, 2016; Trachsel *et al.*, 2011). In this study, the result showed that water deficit in seedling stage had no impact on the root number and root length, but the root activity was significantly affected by water stress. The reason might be related to the low evapotranspiration at seedling stage, and the soil moisture can satisfy the needs of maize growth. Such speculation is related to the jointing stages that deficit irrigation has significantly affected root number and root activity. However,  $D_j$  in jointing stage still has not been influenced the root length. This may be related to the depth of the plastic bucket, which is the major reason for limiting the root elongation. Particularly, the deficit irrigation and N-fertilizer application rate significantly affected root activity and nitrogen uptake. Some researchers showed that water shortage can affect soil nutrient availability and absorption by plant roots, and the combined of soil water and soil nutrient management can realize improvement of water and nutrient use efficiencies under conditions of locally restricted irrigation (Basamba *et al.*, 2006; Jin *et al.*, 2014; Stoop *et al.*, 2007; Thorup-Kristensen, 1993; Zhang *et al.*, 2014). In this study, the dry matter accumulation and nitrogen uptake decreased with the increase of N-fertilizer application rate. This may be related to the limits of nutrient requirements for maize crops (Boote *et al.*, 1996; Plénet & Lemaire, 1999; Wang *et al.*, 2021).

In addition, water deficit in seedling stage of a certain role to promote the growth of root system, increase the root activity. Particularly, nitrogen uptake is highly correlated ( $p < 0.01$ ) with nitrogen use efficiency in this study. The effect of water deficit at different growth stages on root activity was shown that jointing stage is the water demand sensitive period. This may be related to the vigorous growth of crops that needs a lot of water and fertilizer to meet the different organs for expansion (Li *et al.*, 2001). The higher root activity corresponds to the higher dry matter accumulation, shows a correlation of, at most, 0.82 (Table 2). Meanwhile, the dry matter accumulation was significantly ( $p < 0.01$ ) associated with plant height and root length in our study. Dry matter accumulation is an important factor for maize source-sink and leaf senescence, which indicated that a delicate balance exists source and sink at maturity stage (Zhang *et al.*, 2010). Our result indicated that the mean dry matter accumulation decreased with the increase of N-fertilizer application rate on averaging irrigation treatments. We can be sure of that there must exist a maximum value N-fertilizer demand for maize.

## Conclusion

Our PCA results show that the top five are  $F_iN_1$ ,  $D_mN_1$ ,  $D_sN_1$ ,  $D_fN_1$  and  $D_jN_1$ , which means that appropriate N-fertilizer application rate is better than high dose. In the place where water sufficient, it is best to choose full irrigation treatments. In other situations, there is a water shortage area, according to our results, water deficit irrigation during maturity is preferred, and then the seedling stage. The analysis shows an obvious trade-off among the aboveground growth parameters, root growth parameters, water and nitrogen use efficiency of maize in the pot experiment. The present study sheds light on the contributions and impacts of irrigation and N-fertilization practices and provides a basis for evaluating and selecting better management practices for field crops. However, a complete pot experiment with two or three growth stage in deficit irrigation is needed to reflect more information about pot experiments.

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