The effect of water deficits around flowering on grain yield and plant morphology of maize in negative irrigation system

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Abstract

Flowering is drought sensitive, which has been used as the critical phase for drought-tolerant evaluation or screening of maize varieties. It is hard to acquire parallel plants in imprecise irrigation regimes. Hence, a negative-pressure irrigation system was introduced for accurate water control in this study. A pot experiment was performed to explore the responses of three maize hybrids – Zhengdan 958, Danyu 39, and Danyu 405 – to water deficit around flowering. Five irrigation regimes, including well-irrigated treatment (CK), ten days of moderate water deficit since 13 - leaf stage (V13M) or tasseling (VTM), and ten days of severe water deficit since 13-leaf stage (V13S) or tasseling (VTS), were applied. The small values of coefficient of variance in most observed traits demonstrated the feasibility and stability in obtaining parallel plants in the negative-pressure irrigation system. Zhengdan 958 had the least grain yield reduction (4.32 – 23.0%) after water deficits, followed by Danyu 405 (13.5 – 27.0%), and Danyu 39 (13.8 – 34.2%). The less affected kernel number in Zhengdan 958 was mainly a result of slightly decreased ear length and small ear tip-barrenness in water deficit. On the other hand, Zhengdan 958 could maintain a more stable whole plant dry matter under water deficits, benefiting from the less influenced leaf area, plant height, and stem diameter. The reported results highlight that Zhengdan 958 showed a better drought tolerance than Danyu 39 and Danyu 405, which was mainly attributed to more stable kernel number and better morphological performances under water deficits.

Abbreviations

CK – sufficient irrigation
V13M – moderate water deficit (M) at 13-leaf stage (V13)
V13S – severe water deficit (S) at 13-leaf stage (V13)
VTM – moderate water deficit (M) at tasseling stage (VT)
VTS – severe water deficit (S) at tasseling stage (VT)
PVC – polyvinyl chloride
ρ – density of water
g – the acceleration of gravity
h1 – water level set in pressure control tube
h2 – the height difference between the bottom line of the water supply bucket and porous ceramic plate
P_0 – atmospheric pressure
P_1 – the pressure in the pressure-control plexiglass pipe
P_2 – the pressure that plants need to overcome for water absorption
CV – coefficient of variation

Introduction

In maize, the flowering period is critical for kernel setting and subsequent grain yield but is sensitive to water deficit (Robins and Domingo, 1953; Claassen and Shaw, 1970; Schussler and Westgate, 1991). The grain yield reduction induced by water deficit is associated with the alterations of massive traits, e.g., asynchronous flowering (Bolanõs and Edmeades, 1993), infertile ears or kernels (Zinselmeier *et al.*, 1999; McLaughlin and Boyer, 2004a, b; Oury *et al.*, 2016), accelerated leaf senescence (Wolfe *et al.*, 1988), reduced biomass production (Cakir, 2004), etc. Some of these secondary traits have been used as criteria for drought-tolerant variety selection in multi-environment trials (Bolanõs and Edmeades, 1996; Edmeades *et al.*, 1999; Sayadi Maazou *et al.*, 2016).

Compared to kernel weight, kernel number was observed to be more sensitive to water deficit around flowering in maize (Otegui, 1995; Chapman and Edmeades, 1999), comprising rows per ear and kernel number per row. The rows per ear establish once ear differentiation initiation at around 9-leaf stage (Abendroth *et al.*, 2011), which is hardly affected by water deficits around flowering. Thus, kernel losing is mainly caused by decreased kernel number per row in response to water deficit around flowering. Kernel formation is tightly associated with the growth of female organs compared to male organs (Borrás and Vitantonio-Mazzini, 2018; Messina *et al*., 2019). The hampered ear growth has an after effect and shortens the effective ear length for kernel setting (Li *et al*., Submitted). Enhanced tip-barrenness lengths caused by floret and/or ovary failure easily occur under water deficits, which has been regarded as the main limitation for kernel setting (Turc and Tardieu, 2018; Gustin *et al*., 2018; Messina *et al*., 2019). Thus, sustaining kernel number would potentially profit grain yield stabilization in response to water deficit around flowering.

Dry matter production is closely associated with maize grain yield. Shoot biomass can completely recover when maize plants subject to a short period of water deficit at an early stage, resulting in a comparable grain yield with well-irrigated plants (Mansouri-Far *et al*., 2010). Shoot dry matter becomes irreversible and sensitive to water deficit since maize steps into the rapid growth period at around 12-leaf stage, leading to great yield reduction (Cakir, 2004). During maize rapid growth period, the expansion of vegetative organs such as leaves and stems, is largely limited, as the main contributor to aboveground biomass reduction (Cakir, 2004). Accelerated leaf senescence could limit dry matter production and then affect grain filling for later water deficit (NeSmith and Ritchie, 1992; Li *et al*., 2018). In addition, shoot biomass partitioning into grains is of great essential for grain yield. Previous papers emphasized that assimilates that were allocated to the ear and ear growth rate during flowering play a determining role in kernel establishment (Severini *et al*., 2011; Borrás and Vitantonio-Mazzini, 2018). Hence, exploring the response of dry matter production to water deficit can better help to understand the causes for grain yield reduction.

Maize hybrids respond differently to water deficit. Drought-tolerant hybrids tend to have better performances than the common hybrids, like, flowering pattern and grain yield formation, etc. A short and insensitive anthesis-silking interval showed in recent released drought-tolerant maize hybrids, which is beneficial to fertilization and kernel number establishment under water deficit (Sun *et al*., 2019). Ao et al (2020) showed that the greater kernel number and aboveground biomass were the reason for grain yield improvement of drought-tolerant hybrids in coping with water stress initiated at around 14-leaf stage. In China, Zhengdan 958 is one of the elite commercial hybrids, and its planting has been lasting for several decades. Thus, it is of great meaning to explore the advantage of Zhengdan 958 for its predominant place in the Chinese seeding market compared to other hybrids.

Studies concerning the response of maize to water deficit were based on both field and pot experiments. Most field trials achieved water deficits by reducing irrigation time or volume (Moser *et al*., 2006; Mansouri-Far *et al*., 2010). It is more convenient to control soil water condition in pot experiment, but it usually takes several days to reach the target soil moisture (Zinselmeier *et al*., 1999; Echarte and Tollenaar, 2006; Oury *et al*., 2016a). It is hard to provide a “fine-tuning” soil moisture control to minimize plant performance differences through the above irrigation strategies. Thus, it urgently needs a more precise irrigation system for high-level phenotype data collecting. Recently, a negative-pressure irrigation system gradually used in China can accurately and consistently provide water for plant growth (Lei *et al*., 2005; Xu *et al*., 2014). Hence, this system was introduced to achieve precise water management and non-bias comparison among maize hybrids in this study.

The reported research aimed to (i) observe the response of plant morphological performances, grain yield, and yield components in response to 10-days moderate or severe water deficits since 13-leaf stage and tasseling; (ii) compare the performance difference between Zhengdan 958 and the other two maize hybrids (Danyu 39 and Danyu 405) in response to water deficits, and (iii) evaluate the precious of the negative-pressure irrigation system by calculating the coefficient of variation of the observed traits.

Material and methods

Negative-pressure irrigation system

In this study, the negative-pressure irrigation system (Patent No. 201320291701.6 CN) was set up for plant growth, with technical support from the Beijing Academy of Agriculture and Forestry Sciences (Wu *et al*., 2016). As shown in Fig. S1, this system includes the plant growth part, water supply part, pressure control part, and air flow-in part. The plant growth part is a pot with a diameter of 0.25 m and a height of 0.4 m, with a thickness of 15 mm and a diameter of 0.2 m porous ceramic plate placed at the bottom of the pot at a 15–25°inclined angle. The porous ceramic plate is strictly airtight but permeable for water with a cavity inside, which needs to be immersed in water over one week before placing it into the pot. The water supply bucket is a polyvinyl chloride (PVC) tube with a length of 1 m and an inside diameter of 0.15 m, sealed with PVC plugs at both ends. These two parts are connected by a transparent plastic hose shown in Fig. S1. The pressure control part is mainly a pressure-control pipe made of plexiglass, linking to the water supply bucket through PVC pipes. The air flow-in tube is a capillary, which connects to the pressure-control pipe directly. To maintain the pressure of the entire system, all the connections seal with specific PVC glue to avoid air leaking.

The running of this system mainly depends on the negative pressure, which is triggered by soil water potential gradient as plants absorb water. In detail, growing plants continuously absorb water from the soil matrix leading to low water potential in the soil matrix. Subsequently, soil matrix sucks water through porous ceramic plates because of the water potential gradient, resulting in the water level decreasing in the water supply bucket. The pressure in the water bucket decreased because of air volume increased, which drives the air flow in through the pressure-control pipe. Finally, the entrance of air gradually alleviates the pressure in the water supply bucket, and the system re-balanced again. The detailed working theory is as follows.

The design diagram of the system shows in Fig. S1. The water supply bucket is full of water to exhaust air at the beginning, and all the valves except the one installed at the bottom of the water supply bucket are turning off to ensure the tightness of the whole system. The pressure at the junction between capillary and pressure-control plexiglass pipe is equal to atmospheric pressure (P_0) when the pressure of the system reaches equilibrium. The pressure of air in the pressure-control plexiglass is $P_1 = \rho g h1 - P_0$, in which ρ is the density of water, and g is the acceleration of gravity. The pressure at the junction between the water supply bucket and PVC connection pipe is also P_1 because of equilibrium. Although the gravity generated by the height difference between the bottom line of water supply bucket and porous ceramic plate ($h2$) could provide a positive pressure for plants absorbing water from soil matrix, there is still a negative pressure (P_1) from the system that plants need to overcome. Thus, the final pressure plants need to overcome is $P_2 = P_1 - \rho g h2 = \rho g\ (h1-h2) - P_0$. The $h2$ fixes once the system establishes, thus the pressure will change along with alteration of the water level ($h1$) in the pressure-control plexiglass pipe. The water will gradually consume with plant growth in the water supply bucket, and it fills up again whenever water drops down to around 1/4 of the bucket in the process of running.

Experimental site and design

The experiment was conducted at Shangzhuang Experimental Station, China Agricultural University, Beijing, China (40°08′09″ N, 116°10′52″ E). The negative-pressure irrigation system was directly placed on the ground with a plastic shelter to avoid rainfall. The filled soil in pots was the surficial soil (0–20 cm) excavated from the farm field, classified as silt loamy. The organic matter, total nitrogen, available phosphorus, and available potassium of the soil were 20.61 g kg^{-1}, 1.13 g kg^{-1}, 97.58 mg kg^{-1}, 111.60 mg kg^{-1}, respectively. The bulk density and field capacity of the soil were 1.32 g cm^{-3} and 29.60% (w/w), respectively. The filled soil was air-dried first, and then sieved through a fine mesh to remove stones and straw residue. And an equivalent weight of soil (20 kg) was filled into each bucket after evenly mixing with 1.8 g N (0.09 g kg^{-1} N), 2 g P (0.10 g kg^{-1} P), and 3 g K (0.15 g kg^{-1} K) by shovel.

Three maize hybrids (Zhengdan 958, Danyu 39, and Danyu 405) were selected as the materials in this research, all released in the 2000s. Planting was conducted on June 23rd in 2015. Three to four seeds were sown in each pot with a depth of 3–5 cm; two plants in each pot were left at around 3-leaf stage. Top-dressing was processed with 1.2 g N (0.06 g kg^{-1} N) at around 12-leaf stage for each bucket. Five water treatments were designed by adjusting $h1$ of the negative-pressure irrigation system according to Xu et al (2014), including (1) sufficient irrigation treatment (CK) with $h1$ setting at 0.50m (approximate 70–80% field capacity) during the whole maize growing season, (2) ten days of moderate water deficit since 13-leaf stage (V13M) ($h1$ = 0.75 m, approximate 50–60% filed capacity), (3) ten days of moderate water deficit since tasseling stage (VTM), (4) ten days of severe water deficit since 13-leaf stage (V13S) ($h1$ = 0.95 m, 30 – 40% filed capacity), and (5) ten days of severe water deficit since tasseling stage (VTS). To guarantee better pollination for plants, daily artificial pollination was conducted at around noon since the 1st days of silk emergence. Plants were harvested after physiological maturity.

Determination of morphological indicators, grain yield and yield components

Plant height, stem diameter and leaf area

Non-destructive measurement was conducted to determine plant height, stem diameter, and leaf area with a 5-day interval before and during water treatment in July 7th-Aug 6th, and one more time at the end of water treatment. Six representative plants in each hybrid were selected for measurement before water deficit onset, while three plants in each treatment were selected for measurement during and after water deficits. Plant height (cm) was the distance from the soil surface to the tip of the top leaf, determined by the steel tape. Plant diameter (mm) was determined by measuring the width of the 2nd internode from the base, using an electric caliper. The leaf area was calculated using the

Fig. 1 -

Table 1 - The differences in ear diameter, ear length, ear tip-barrenness length, whole plant dry matter, rows per ear, kernel number per plant, 100-kernel weight, and grain yield among water treatments and maize hybrids (*Zhengdan* 958, *Danyu* 39, and *Danyu* 405). CK, well-irrigated treatment; V13M, ten days (Aug 10^{th}-20^{th}) of moderate water deficit initiated at 13-leaf stage; V13S, ten days (Aug 10^{th}-20^{th}) of severe water deficit initiated at 13-leaf stage; VTM, ten days (Aug 14^{th}-24^{th}) of moderate water deficit initiated at tasseling; VTS, ten days (Aug 14^{th}-24^{th}) of severe water deficit initiated at tasseling. Values without same lowercase letters mean significant difference at P < 0.05 among water treatments within hybrids, while values without different uppercase letters mean significant difference at P < 0.05 among maize hybrids. "*", "", and "***" mean significant difference at the levels of P < 0.05, 0.01 < P < 0.05, and P < 0.001, respectively. "ns" means no significant difference.**

Maize Variety	Water treatment	Whole plant dry matter	Ear length / cm	Tip-barrenness length /cm	Ear diameter / mm	Rows per ear	Kernel number $plant^{-1}$	100-Kernel weight / g	Grain yield per plant / g
Zhengdan 958	CK	365 a	17.5 a	0.733 c	50.5 a	13.7 b	491 a	28.4 a	139 a
	V13M	354 a	17.6 a	1.82 b	49.7 a	14.7 ab	481 ab	27.7 a	133 a
	V13S	305 b	16.0 bc	2.95 a	49.8 a	14.3 ab	409 b	26.6 a	107 b
	VTM	341 ab	16.8 ab	2.35 ab	49.0 a	15.0 a	451 ab	26.6 a	121 ab
	VTS	306 b	15.5 c	2.32 ab	49.1 a	15.0 a	424 ab	25.7 a	109 b
	Mean	**334 B**	**16.7 B**	**2.03 B**	**49.6 C**	**14.5 B**	**451 B**	**26.9 B**	**122 A**
Danyu 39	CK	398 a	18.9 a	2.47 b	56.9 a	15.0 a	459 a	32.3 a	152 a
	V13M	365 abc	16.7 b	3.93 a	57.5 a	15.7 a	415 a	31.9 ab	131 ab
	V13S	326 c	17.0 b	4.07 a	56.2 a	15.0 a	403 a	30.3 ab	114 ab
	VTM	392 ab	17.3 ab	3.98 a	58.3 a	16.0 a	380 a	31.3 ab	127 ab
	VTS	336 bc	16.8 b	4.88 a	54.0 a	14.7 a	349 a	28.8 b	100 b
	Mean	**363 A**	**17.4 AB**	**3.90 A**	**56.4 A**	**15.3 B**	**401 C**	**31.0 A**	**125 A**
Danyu 405	CK	452 a	19.0 a	2.70 b	56.0 a	17.7 a	557 a	26.5 a	148 a
	V13M	405 ab	18.3 ab	3.55 ab	54.9 ab	17.3 a	479 ab	27.0 a	128 ab
	V13S	307 d	16.6 b	4.43 a	53.3 b	17.0 a	450 b	25.4 a	115 b
	VTM	377 bc	17.2 ab	3.85 a	52.8 b	17.7 a	468 ab	27.2 a	127 ab
	VTS	329 cd	17.1 b	4.98 a	53.4 b	17.7 a	414 b	26.0 a	108 b
	Mean	**374 A**	**17.6 A**	**3.90 A**	**54.1 B**	**17.5 A**	**473 A**	**26.4 B**	**125 A**
ANOVA									
Variety		**	ns	***	***	***	***	***	ns
Water supply		***	**	***	ns	ns	***	***	***
Variety × Treatment		ns	ns	ns	ns	ns	*	ns	**

method mentioned in Pearce et al (1975), *i.e.* plant leaf area was the sum of individual leaf areas calibrated with a coefficient of 0.75 for fully expanded leaves and 0.5 for visible leaves (coefficient × length × width).

Shoot dry matter, grain yield, and yield component

At harvest, three representative plants in each treatment were detached and then oven-dried at 80°C till constant weight for shoot dry matter. The rest plants in each treatment were sampled for the determination of other indicators including ear diameter, ear length, ear tip-barrenness length, rows per ear, kernel number per plant, 100-kernel weight, and grain yield. Kernel number was the amount of the swelled kernels with the exclusion of the shriveled kernels. Grain yield was determined by the oven-dried kernel weight threshed from the whole ear, with calibration of 14% moisture content. And the 100-kernel number were transferred through grain yield and kernel number per ear.

Data analysis

Analysis of variance (ANOVA) was conducted in SPSS 18.0, with maize hybrids, water deficit treatments, and their interaction as the source of variation. Maize hybrids and water deficit treatments and their interaction were regarded as the fix factors, while replications as the random factor. Fisher`s least significant difference method programmed in SAS 9.0 was used for multiple comparisons for traits among hybrids and water treatments, while $LSD_{0.05}$ was used as the scale for significant difference analysis. All the figures were produced in SigmaPlot 12.5. The coefficient of variation (CV, %) was calculated by dividing the standard deviation by its corresponding mean values.

Results and Discussion

Biomass production and ear characteristics

Hybrid and water treatment both had significant effects on the whole plant dry matter but their interaction did not (Table 1). The whole plant dry matter remarkably decreased by 15.6–32.1% in severe water deficits at both 13-leaf stage (V13S) and tasseling stage (VTS), but was slightly or not influenced in moderate water deficit initiated at 13-leaf stage (V13M) and tasseling stage (VTM), as compared to well irrigated treatment (CK). The sustained low shoot biomass in water deficit at the reproductive stage could render to kernel losing and yield reduction (Schussler and Westgate, 1991; Pandey *et al.*, 2000a; b; Cakir *et al.*, 2004; Moser *et al.*, 2006; Mi *et al.*, 2018). However, restored shoot biomass was observed in early water deficit at the vegetative stage, conferring a comparable grain yield with well-watered plants (Mansouri-Far *et al.*, 2010). Thus, resilient shoot biomass was associated with the stationary of grain yield. The whole plant dry matter of Zhengdan 958 could compare to that of the other two hybrids in V13S and VTS, even though the whole plant dry matter was the lowest in V13M, VTM and CK (Table S1). Furthermore, the whole plant dry matter of Zhengdan 958 had low decreased percentages in water deficits (Table S2). This indicated that the whole plant dry matter of Zhengdan 958 was more resilient in water deficit, which could be an advantage for its drought-tolerant ability.

Significant difference was observed in ear diameter and ear tip-barrenness length among maize hybrids. Water treatments remarkably influenced ear length and tip-barrenness length. There was no hybrid and treatment interaction effect on all the ear characteristics (Table 1). The ear lengths significantly decreased in severe water deficits (V13S and VTS), but was slightly or not influenced in moderate water deficit (V13M and VTM) as compared to well irrigated treatment (CK). On the contrary, the tip-barrenness lengths were similar among V13S, VTM, and VTS treatments, but were slightly or not influenced by V13M treatment as compared to CK. Both decreased ear lengths and increased tip-barrenness lengths reduced the effective ear lengths, explaining the decreased kernel number under water deficit (Li *et al.*, Submitted). There were no significant differences in ear diameters among all treatments in both Zhengdan 958 and Danyu 39, while ear diameters significantly decreased in V13S, VTM, and VTS compared to CK in Danyu 405. Regarding hybrids, Zhengdan 958 hold the smallest ear length, ear diameter, and tip-barrenness length. Danyu 405 had the greatest ear length and Danyu 39 had the greatest ear diameter, both of which hold comparable tip-barrenness lengths.

Yield components and grain yield

Hybrid had significant effects on rows per ear and yield components (kernel number per plant and 100-kernel weight) but not on grain yield; water treatment had more significant effects on both yield and yield components except for rows per ear (Table 1). The interaction between variety and water treatment had weak or no significant effects on yield and yield components. There was no decrease in rows per ear in water deficit treatments as compared to CK. Thus, the decreased kernel number in water deficit might be attributed to the shortened ear length (Li *et al.*, Submitted), failed fertilization (Oury *et al.*, 2016), and kernel abortion (McLaughlin and Boyer, 2004a, b; Shen *et al.*, 2020). Additionally, 100-kernel weight was slightly or not influenced by water deficit in three hybrids except for VTS in Danyu 39 (Table 1). Among hybrids, Danyu 405 had the most rows per ear, likely resulting in a higher kernel number per plant. Kernel

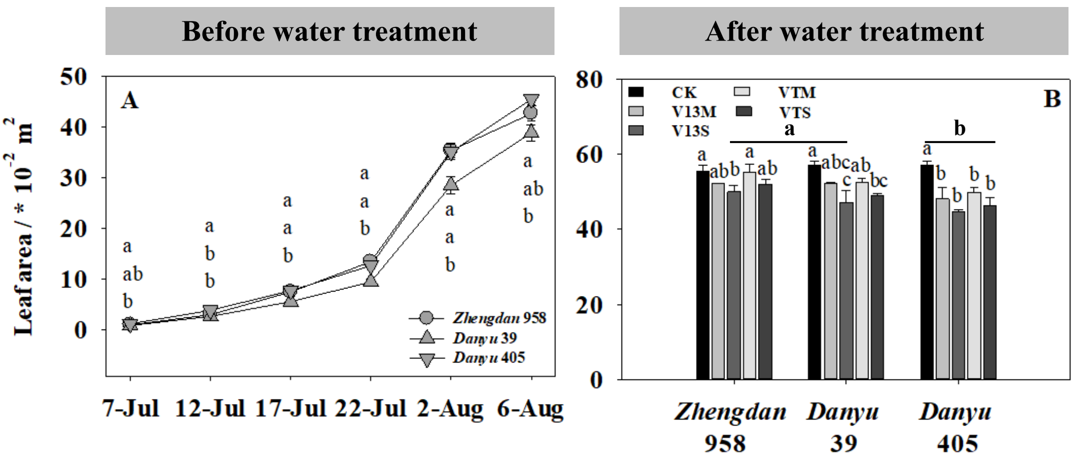

Fig. 1 - Leaf areas of three maize hybrids (*Zhengdan* 958, *Danyu* 39, and *Danyu* 405) measured before water treatment on July 7th, 12th, 17th, and 22nd, and Aug 2nd, and 6th (A), and after water treatment at around tasseling (B). CK, well-irrigated treatment; V13M, ten days (Aug 10th-20th) of moderate water deficit initiated at 13-leaf stage; V13S, ten days (Aug 10th-20th) of severe water deficit initiated at 13-leaf stage; VTM, ten days (Aug 14th-24th) of moderate water deficit initiated at tasseling; VTS, ten days (Aug 14th-24th) of severe water deficit initiated at tasseling. Values without same letters mean significant differences at P < 0.05. Bars stand for standard errors.

Table 2 - The comparison of coefficient of variance (CV) among maize hybrids (*Zhengdan* 958, *Danyu* 39, and *Danyu* 405), also water treatments (CK, V13M, V13S, VTM, and VTS). CK, well-irrigated treatment; V13M, ten days (Aug 10th-20th) of moderate water deficit initiated at 13-leaf stage; V13S, ten days (Aug 10th-20th) of severe water deficit initiated at 13-leaf stage; VTM, ten days (Aug 14th-24th) of moderate water deficit initiated at tasseling; VTS, ten days (Aug 14th-24th) of severe water deficit initiated at tasseling. Multiple comparisons were separately conducted among maize hybrids and water treatments, and values without same letters mean significant difference at P < 0.05.

Traits	Comparison of coefficient of variance (CV) among maize hybrids			Comparison of coefficient of variance (CV) among water treatments				
	Zhengdan 85	Danyu 39	Danyu 405	CK	V13M	V13S	VTM	VTS
Ear length	4.05% b	5.65% b	8.36% a	7.70% a	5.54% a	6.84% a	5.04% a	4.99% a
Ear tip-barrenness length	30.6% a	17.2% a	13.5% a	26.4% a	18.6% a	18.5% a	23.7% a	14.9% a
Ear diameter	2.72% a	4.45% a	2.30% a	5.84% a	1.82% a	4.04% a	2.02% a	2.09% a
Shoot dry weight	6.69% a	8.39% a	6.81% a	8.15% a	5.30% a	9.63% a	6.56% a	6.85% a
Kernel number per plant	2.69% a	3.65% a	2.87% a	1.23% a	3.98% a	4.12% a	3.52% a	2.51% a
100-Kernel weight	3.44% a	2.54% a	3.35% a	1.63% a	3.79% a	3.14% a	3.67% a	3.31% a
Grain yield per plant	1.92% a	4.06% a	2.85% a	2.52% b	3.09% ab	5.28% a	1.10% b	2.75% ab

number per ear of Danyu 39 was significantly lower than that of Zhengdan 958, even though they had comparable rows per ear. The 100-kernel weight was higher in Danyu 39 than in Zhengdan 958 and Danyu 405. Additionally, water deficit had the lowest effect on kernel number in Zhengdan 958 and on 100-kernel weight in Danyu 405, as shown in Table S1 and S2.

A resilient kernel number rather than kernel weight is more conducive to the stabilization of grain yield under these water deficits (Claassen and Shaw, 1970; Otegui, 1995; Chapman and Edmeades, 1999). The whole plant dry matter of Zhengdan 958 had a greater buffering capacity in response to water deficit, which might be beneficial for grain yield maintenance as grain yield was a result of transformation of accumulated dry matter into grains (Jurgens *et al.*, 1978). Grain yield of later water deficits (average of VTM and VTS) was significantly lower than that of early water deficits (average of V13M and V13S). However, there was no significant difference in the whole plant dry matter between these two groups of water deficits (Table 1). The low grain yield in later water deficits could be explained by reduced assimilate supply into kernels and kernel abortion (Schussler and Westgate, 1991).

Leaf area, plant height, and stem diameter

Leaf area

Hybrids showed different values in leaf area before water deficit. Zhengdan 958 and Danyu 405 had similar leaf areas, while Danyu 39 had the lowest leaf area (Fig. 1A). After water deficit application, the averaged leaf area across all treatments of Zhengdan 958 was still similar to that of Danyu 39, and significantly greater than that of Danyu 405 (Fig 1B). However, leaf areas of hybrids decreased with increased water deficit severity (Fig 1B), demonstrating different susceptibilities of hybrids to water deficits. Leaf area significantly decreased by 9.82% in V13S in Zhengdan 958, by 17.4% in V13S, and by 14.3% in VTS in Danyu 39. The reduction in leaf area showed in an order of Zhengdan 958 < Danyu 39 < Danyu 405 in all water deficits (Table S2). Thus, the leaf area of Zhengdan 958 was most tolerant to water deficits, which could also be one reason for the higher water deficit resistance.

Plant height

Plant heights of hybrids behaved differently before water deficit (Fig. 2A1). In detail, the plant heights of Zhengdan 958 were similar to that of Danyu 405 and significantly higher than that of Danyu 39 before Jul 22nd, whereas they were similar to that of Danyu 39 and lower than that of Danyu 405 on Aug 2nd and 6th (Fig. 2A1). After water deficit application, the averaged plant height across all treatments of Danyu 405 was the highest (ca. 268 cm), while that of Zhengdan 958 was the lowest (ca. 248 cm) (Fig. 2A2). Plant heights of Zhengdan 958 only significantly decreased by 7.87% in V13M and by 12.1% in V13S in comparison with CK (ca. 258 cm). Plant heights of Danyu 39 decreased in all water deficits in comparison with CK (ca. 296 cm), with reductions of 14.7% in V13M, 17.9% in V13S, 12.7% in VTM, and 17.8% in VTS. Whereas, plant heights of Danyu 405 remarkably decreased by water deficits except for VTM (ca. 281 cm), with reductions of 6.55% in V13M, 8.63% in V13S, and 5.93% in VTS in comparison with CK. Additionally, plant height of Zhengdan 958 was the lowest in CK and V13M, but was comparable to that of Danyu 39 in V13S and VTM and even that of Danyu 405 in VTS (Table S1). Furthermore, plant heights of Zhengdan 958 showed the least decreased percentages in VTM and VTS (Table S2).

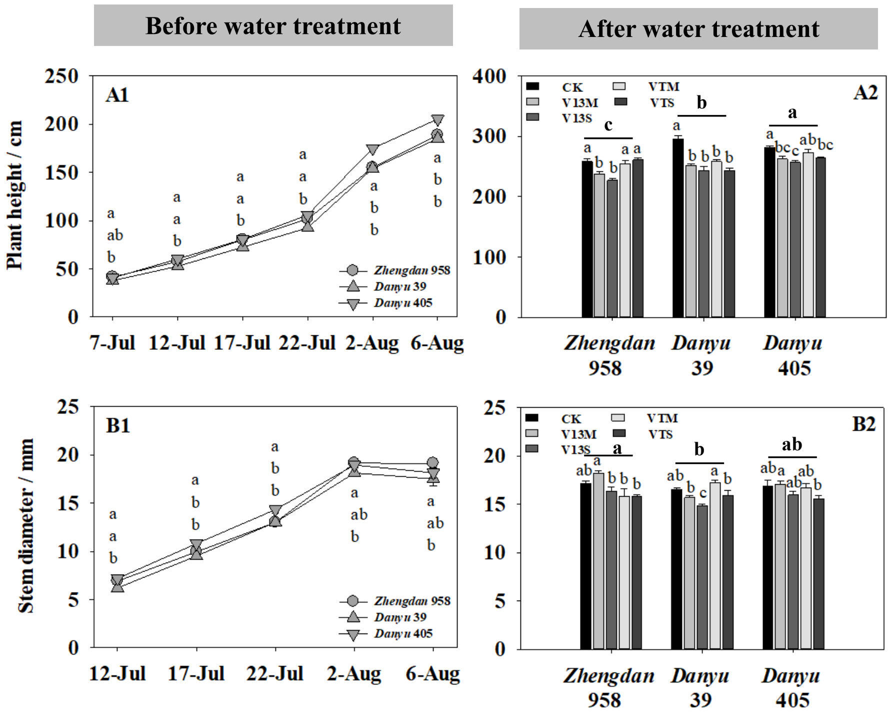

Fig. 2 - Plant heights (A) and stem diameters (B) measured before (A1 and B1) and after (A2 and B2) water treatment. CK, well-irrigated treatment; V13M, ten days (Aug 10th-20th) of moderate water deficit initiated at 13-leaf stage; V13S, ten days (Aug 10th-20th) of severe water deficit initiated at 13-leaf stage; VTM, ten days (Aug 14th-24th) of moderate water deficit initiated at tasseling; VTS, ten days (Aug 14th-24th) of severe water deficit initiated at tasseling. Values without same letters mean significant differences at $P < 0.05$. Bars stand for standard errors.

Stem diameter

Before water deficit, Danyu 39 had the smallest stem diameter among three hybrids, whereas Danyu 405 had the largest stem diameters on Jul 12th, 17th, and 22nd (Fig. 2B1). After water deficit application, the averaged stem diameters across all treatments was in an order of Zhengdan 958 > Danyu 405 > Danyu 39 (Fig. 2B2). The stem diameters were not influenced by water deficits in Zhengdan 958 and Danyu 405, but were significantly decreased by V13S in Danyu 39 (Fig 2B2). Additionally, the stem diameters of Zhengdan 958 were similar to that of the other two hybrids in VTM, VTS and CK, while they were the greatest in V13M and V13S (Table S1). Furthermore, the stem diameters of Zhengdan 958 decreased the least in V13S and V13M (Table S2), indicating that the stem diameter of Zhengdan 958 had a strong drought tolerant ability during this period.

Overall, Zhengdan 958 had better performances in yield and plant morphology in response to water deficits, resulting in a high drought resistance. The three maize hybrids (Zhengdan 958, Danyu 39, and Danyu 405) used in this study were all released during 2000s in China, which all had been documented as drought-tolerant maize hybrids (Cheng, 2010; Tang *et al.*, 2012). However, only Zhengdan 958 is still dominated in Chinese maize seeding market until now. The above results gave the evidences that Zhengdan 958 was more tolerant to water deficit than Danyu 39 and Danyu 405. Also, there were many comparisons conducted between Zhengdan 958 and many other maize hybrids, showing that Zhengdan 958 was not only stand out among peers but also could be comparable to the later released hybrids (Pei *et al.*, 2019; Hao *et al.*, 2019). Drought tolerance should be one reason.

The precision of the negative irrigation system-Coefficient of variance

The precision of the negative-pressure irrigation system was evaluated by the coefficient of variance (CV) of the observed indicators in this study, as showed in Table 2 and Fig. 3. The CVs varied greatly in ear tip-barrenness length ranging from 13.5 to 30.6%, while they were around or below 10% for other ear characteristics, shoot dry weight, yield and yield components (Table 2 and Fig. 3). Furthermore, there was a decreasing trend of CVs with the plant growing, as shown in Fig. 3. The stable behaviors of the indicators could be mainly attributed to the stable water supplication of this system, which had been documented as a consecutive and stable water supplication with a narrow fluctuation (less than 5%) in soil water content by using this system (Li *et al.*, 2017 a, 2019). This irrigation strategy is also beneficial for resource use efficiency (Li *et*

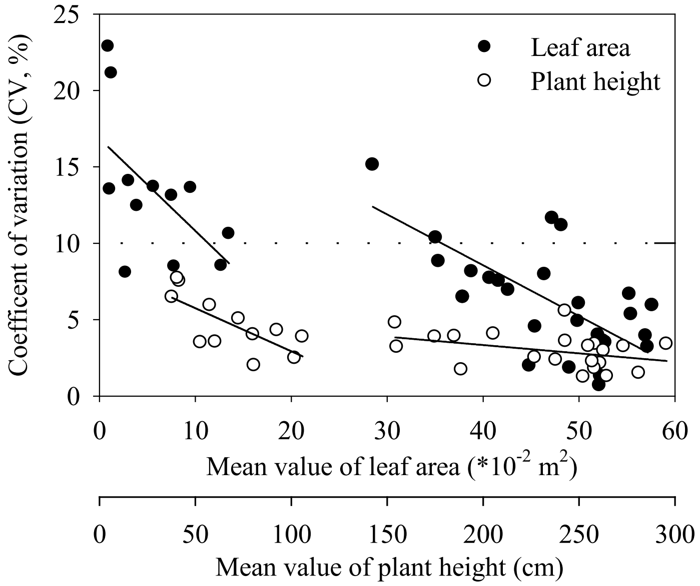

Fig. 3 - The correlation of coefficient of variance (CV) with the mean value of leaf area and plant height. Each point represents one treatment of one maize hybrid at a specific sampling date. The scatters of both traits were non-deliberately divided into two groups by the gap, and then linear regressions were fitted separately among different groups.

al., 2017 a, 2019; Gao *et al.*, 2019 a, b) and plant production (Li *et al.*, 2017 b, c). It had been used in massive species, like, crops, vegetables, orchard, and tobacco in practice. Thus, more precise soil moisture with small gradient can be set up with this system, which is benefit to determining threshold of water stress for different plant species. Recently, a high-throughput phenotype theory and the application are pouring in agronomy, but still not widely used in China (Hu *et al.*, 2019; Xu *et al.*, 2020). Thus, negative irrigation system could be equipped in a high-throughput platform for accurately controlling water to meet requirements for high-quality researches.

Conclusions

In the present study, the most insensitive indicators were ear diameter, rows per ear, and 100-kernel weight when maize suffered water deficits around flowering. Whereas, kernel number and the whole plant dry matter were more sensitive especially under severe water deficits, which could be the main reasons for grain yield reduction. Water deficit-induced decreased kernel number might be the result of decreased ear length and increased tip-barrenness length, while the decreased whole plant dry matter might be associated with the influenced leaf areas and stem expansion. Furthermore, Zhengdan 958 showed less influence in most indicators, which could be the explanation for its stronger drought-tolerant ability than the two other maize hybrids. Ultimately, the successfully distinguishable plant performances among hybrids and treatments were largely attributed to the effective water controlling of the negative pressure system used in this study.

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Table S1 - The difference in the whole plant dry matter, grain yield, kernel number per plant, 100-kernel weight, leaf area, plant height, and stem diameter among maize hybrids (*Zhengdan* 958, *Danyu* 39, and *Danyu* 405) under each water treatment. CK, well-irrigated treatment; V13M, ten days (Aug 10th-20th) of moderate water deficit initiated at 13-leaf stage; V13S, ten days (Aug 10th-20th) of severe water deficit initiated at 13-leaf stage; VTM, ten days (Aug 14th-24th) of moderate water deficit initiated at tasseling; VTS, ten days (Aug 14th-24th) of severe water deficit initiated at tasseling. Values without same letters mean significant differences at P < 0.05.

Water treatment	Maize Variety	Whole plant dry matter	Grain yield per plant / g	Kernel number plant^{-1}	100-Kernel weight / g	Leaf area / * 10^{-2} m^2	Plant height / cm	Stem diameter / mm
CK	*Zhengdan 985*	365 b	139 b	491 b	28.4 b	55.4 a	258 b	17.1 a
	Danyu 39	398 ab	152 a	459 c	33.0 a	57.2 a	296 a	16.6 a
	Danyu 405	452 a	148 a	557 a	26.6 c	57.0 a	281 a	16.9 a
V13M	*Zhengdan 985*	354 b	133 a	481 a	27.7 b	52.1 a	238 b	18.2 a
	Danyu 39	365 ab	131 a	415 b	31.6 a	52.2 a	252 a	15.7 c
	Danyu 405	405 a	128 a	479 a	26.9 b	48.2 a	263 a	17.1 b
V13S	*Zhengdan 985*	305 a	107 a	409 b	26.2 b	50.0 a	227 b	16.4 a
	Danyu 39	326 a	114 a	380 b	30.0 a	47.2 a	243 ab	14.8 b
	Danyu 405	307 a	115 a	450 a	25.6 b	44.8 a	257 a	16.0 ab
VTM	*Zhengdan 985*	341 b	121 b	451 a	26.8 b	55.3 a	255 b	15.9 a
	Danyu 39	392 a	127 a	403 b	31.4 a	52.5 a	258 b	17.2 a
	Danyu 405	377 ab	127 a	468 a	27.1 b	50.0 a	273 a	16.7 a
VTS	*Zhengdan 985*	306 a	109 a	424 a	25.7 b	52.0 a	261 a	15.8 a
	Danyu 39	336 a	100 b	349 b	28.8 a	49.0 ab	243 b	15.9 a
	Danyu 405	329 a	108 a	414 a	26.0 b	46.4 b	265 a	15.6 a

Table S2 - The decreased percentages of the whole plant dry matter, grain yield, kernel number per plant, 100-kernel weight, leaf area, plant height, and stem diameter of maize varieties (*Zhengdan* 958, *Danyu* 39, and *Danyu* 405) under each water deficit treatment, compared to that in well-irrigated treatment (CK). V13M, ten days (Aug 10th-20th) of moderate water deficit initiated at 13-leaf stage; V13S, ten days (Aug 10th-20th) of severe water deficit initiated at 13-leaf stage; VTM, ten days (Aug 14th-24th) of moderate water deficit initiated at tasseling; VTS, ten days (Aug 14th-24th) of severe water deficit initiated at tasseling.

Water treatment	Maize Variety	Whole plant dry matter	Grain yield per plant / g	Kernel number plant^{-1}	100-Kernel weight / g	Leaf area / * 10^{-2} m^2	Plant height	Stem diameter
CK	*Zhengdan 985*	——	——	——	——	——	——	——
	Danyu 39	——	——	——	——	——	——	——
	Danyu 405	——	——	——	——	——	——	——
V13M	*Zhengdan 985*	3.01%	4.32%	2.04%	2.46%	5.96%	7.75%	-6.43%
	Danyu 39	8.29%	13.82%	9.59%	4.24%	8.74%	14.86%	5.42%
	Danyu 405	10.40%	13.51%	14.00%	-1.13%	15.44%	6.41%	-1.18%
V13S	*Zhengdan 985*	16.44%	23.02%	16.70%	7.75%	9.75%	12.02%	4.09%
	Danyu 39	18.09%	25.00%	17.21%	9.09%	17.48%	17.91%	10.84%
	Danyu 405	32.08%	22.30%	19.21%	3.76%	21.40%	8.54%	5.33%
VTM	*Zhengdan 985*	6.58%	12.95%	8.15%	5.63%	0.18%	1.16%	7.02%
	Danyu 39	1.51%	16.45%	12.20%	4.85%	8.22%	12.84%	-3.61%
	Danyu 405	16.59%	14.19%	15.98%	-1.88%	12.28%	2.85%	1.18%
VTS	*Zhengdan 985*	16.16%	21.58%	13.65%	9.51%	6.14%	-1.16%	7.60%
	Danyu 39	15.58%	34.21%	23.97%	12.73%	14.34%	17.91%	4.22%
	Danyu 405	27.21%	27.03%	25.67%	2.26%	18.60%	5.69%	7.69%

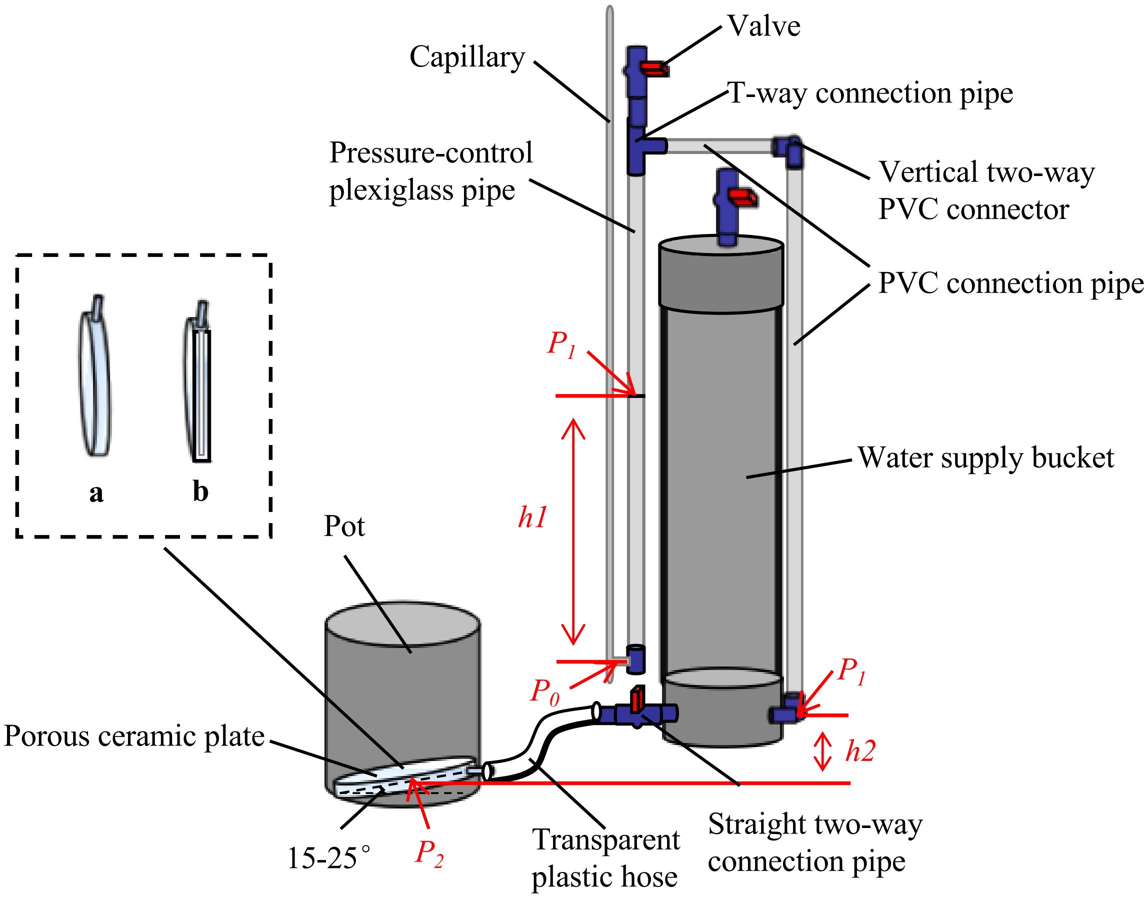

Fig. S 1 - Design diagram of the negative-pressure irrigation system (Patent No. 201320291701.6 CN). a, surface of the porous ceramic plate; b, inner structure of the porous ceramic plate; *h1*, water level set in pressure control tube; *h2*, the height difference between the bottom line of the water supply bucket and porous ceramic plate. P_0, atmospheric pressure; P_1, the pressure in the pressure-control plexiglass pipe; P_2, the pressure that plants need to overcome for water absorption. The 15-25° is the tilt angle of the porous ceramic plate inside the pot.