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RESEARCH ARTICLE

Effect of a new antitranspirant on the physiology and water use efficiency of soybean under different irrigation rates in an arid region

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Abstract Antitranspirants are exogenous substances applied to leaves to reduce luxury transpiration by regulating stomatal conductance to increase water use efficiency (WUE). A cheap and environmentally-friendly antitranspirant, FZ, was newly developed, extracted mainly from *Alhagi sparsifolia*. Its effects on soybean water use were investigated in a field experiment using the locally-used irrigation rate and a low irrigation rate (The lower and upper limit of irrigation is 40%–70% of field capacity). Foliar application of FZ and measurement of leaf physiological characteristics, final biomass, seed yield and water use efficiency were carried out during the pod bearing and pod filling stages of drip-irrigated soybean with film-mulching. Under the low irrigation rate, leaf stomatal conductance (g_s) and transpiration rate (Tr) decreased significantly by 7 d after spraying, but photosynthesis (Pn) and instantaneous water use efficiency (WUE_{in}) were not significantly affec ted. The stomatal frequency, stomatal aperture, g_s, Tr and Pn decreased by 1 d after spraying, without significantly increasing WUE_{in}. However, applying FZ during the pod bearing and pod filling stages did not significantly affect the final biomass, water consumption, seed yield and WUE of soybean. Under the locally-used irrigation rate, applying FZ increased the activities of superoxide dismutase and peroxidase in the leaves by 38% and 33%, respectively, but did not significantly affect g_s, Tr, Pn, stomatal aperture and stomatal frequency. Applying FZ three times during pod bearing and pod filling stages enhanced seed yield and WUE by 24% and 21%, respectively, but did not significantly affect the final biomass and water consumption. Therefore, seed yield and WUE of soybean were significantly increased by foliar application of FZ during the pod bearing and pod filling stages under the locally-used irrigation rate in arid region, but applying FZ did not have a positive effect on water use efficiency of soybean under a low irrigation rate.

Keywords antitranspirant, soybean, water deficit, leaf gas exchange, enzymes activities, water consumption, seed yield

1 Introduction

Reducing crop luxury transpiration is important in improving water productivity[1]. The sensitivity of leaf photosynthesis (Pn) and transpiration (Tr) to stomatal conductance (g_s) differs. Leaf Pn and Tr increase with increasing g_s, but when leaf g_s reaches a certain value, Pn does not obviously increase further but Tr increases linearly[2], so crop luxury transpiration may occur. A balance between leaf photosynthesis and transpiration can be achieved by adjusting the stomatal behavior to the optimal status using exogenous substances (antitranspirants), which lead to an increase in water use efficiency (WUE) at the leaf level. Also, antitranspirants can increase leaf superoxide dismutase (SOD) and peroxidase (POD) activities to improve drought resistance in crops[3–8], and alleviate the negative effects of drought on crop production[9–14].

The antitranspirants include phenylmercuric acetate (PMA)[15–26], salicylic acid (SA)[4,6,8,15,27–29], abscisic acid (ABA)[3,5,9,15,16,30–40], kaolin[14,24,41–44], Vapor Gard[39,45–48] and some others. The effect of an antitranspirant is influenced by its chemistry, soil moisture conditions and spraying period[6,9,10,12,37,48–54]. Under a low irrigation rate, spraying kaolin on the grape significantly reduced leaf g_s and Tr, and improved leaf WUE, while there are no significant effect under a high irrigation rate[14]. Spraying ABA at different growth stages has different effects on the grain yield of wheat. Spraying

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ABA at the flowering stage significantly increased grain yield but there was no significant effect on grain yield when ABA was applied at the grain filling stage[9,38].

Although PMA, SA and ABA had better control of stomatal behavior[17,28,38], there was a negative effect of PMA and SA on leaves[15,19,22]. And ABA is uneconomic to apply in commercial production due to the high price[5,15]. In this study, alkaloid essential oil was extracted from *Alhagi sparsifolia*, a drought-resistant plant widely distributed in the desert area, and then elements such as zinc, boron, nitrogen, phosphorus and potassium were added to make a cheap and environmentally-friendly antitranspirant, FZ (Xinjiang Huitong Humic Acid Co., Ltd., China).

Agriculture in arid regions largely depends on irrigation because of the shortage of water resources[55,56], and the crop is often subjected to drought stress during the growth period. It is hypothesized that foliar application of FZ can produce positive effects on water use efficiency of soybean in an arid region. To determine the effects of FZ, two irrigation rates were employed in this study: the locally-used irrigation rate and a low irrigation rate. The objectives were to investigate the effects of spraying FZ from the pod bearing stage to pod filling stage on leaf g_s, Pn, Tr, SOD and POD, seed yield and water use efficiency of soybean under different irrigation rates, and then evaluate the effects of FZ on drip irrigated soybean in the arid region, to provide a scientific basis for application of FZ.

2 Materials and methods

2.1 Experimental site and materials

A field experiment was conducted during May to September, 2013 at Shiyanghe Experimental Station for Water-saving in Agriculture and Ecology of China Agricultural University (37°52′ N, 102°50′ E, 1581 m) in Gansu Province in Northwest China. The site has a typical temperate continental climate, with favorable light and temperature, a large difference between day and night temperatures, over 3000 h of average annual sunshine duration, 150 frost-free days, and over 3550 degree-day of annual accumulated temperature (over 0°C), while annual precipitation is 164.5 mm and annual pan evaporation is 2000 mm from 1951 to 2013[57]. The soil texture is a sandy loam to a depth of 0.6 m, with a mean soil dry bulk density of 1.48 $g \cdot cm^{-3}$ and soil water content at field capacity of 0.26 $m^3 \cdot m^{-3}$, respectively. *Glycine max* cv. Zhonghuang 30 is widely planted in the area. The active ingredients and contents of FZ are shown in Table 1. Potassium, nitrogen, phosphorus are the main nutrient elements for growth and development of soybean. The glycinebetaine and proline improves photosynthesis activity, nitrogen fixation and plant stress tolerance[7,58], and abscisic acid (ABA) induces stomatal closure[30].

Table 1 The main constituents of FZ

Ingredient	Content
Potassium/$(mg \cdot g^{-1})$	70.6
Nitrogen/$(mg \cdot g^{-1})$	4.2
Phosphorus/$(mg \cdot g^{-1})$	0.13
Calcium/$(\mu g \cdot g^{-1})$	60
Glycinebetaine/$(mg \cdot mL^{-1})$	9.2
Proline/$(mg \cdot mL^{-1})$	0.5
Abscisic acid/$(\mu g \cdot mL^{-1})$	2.25

2.2 Experimental design

The field experiment was conducted with two irrigation rates, i.e., the locally-used irrigation rate (W1) and a low irrigation rate (W2). No irrigation was applied during the maturation period. During other growth stages, W1 and W2 were irrigated on the same day when the soil water content in W2 reached about 40% of field capacity. The irrigation amounts for W1 and W2 were determined by the upper limits set at 90% and 70%, respectively. The irrigation amount during each growth stage is shown in Table 2. Antitranspirant FZ, or an equal amount of water (CK), were sprayed on W1 and W2. Four treatments were applied with three replicates each, with a total of 12 plots randomly arranged. FZ was evenly sprayed on both sides of soybean leaves at a concentration of 3 $g \cdot L^{-1}$ applying 10 L per plot. FZ was applied during the pod bearing stage (21 July), early pod filling stage (8 August) and late pod filling stage (24 August) at about 8 pm.

Each plot was 25 m^2 (5 m × 5 m) with 1 m wide buffers between each plot. The soybean crop was irrigated by a drip irrigation system under film mulching. The drip irrigation lines (interal inlay drip tape, drip emitters 30 cm, flow rate 2 $L \cdot h^{-1}$, thickness 0.2 mm, diameter 16 mm, Dayu Water Group Co., Ltd.) were 1 m apart. A white

Table 2 Irrigation amount at different growth stages of soybean (mm)

Treatment	Vegetative growth stage		Reproductive growth stage			
	Establishment (05/12–05/21)	Branching (05/21–06/26)	Flowering (06/26–07/21)	Pod bearing (07/21–08/08)	Pod filling (08/08–09/02)	Maturity (09/02–09/18)
W2	–	35	45	50	50	–
W1	–	50	75	70	75	–

Note: Sowing, 10 May; 90 mm irrigation for all treatments for seedling emergence on 10 May; no irrigation was applied from September of previous year.

polyethylene film (90 cm wide, 0.008 mm thick) was laid over each drip irrigation line to reduce evaporation. The drip line was at the center of each plastic film, with 10 cm bare soil between the strips of plastic films. Two rows of soybean were planted per strip of film, at 20 cm from the drip line and 25 cm from the edge of the film. The planting density was 250000 plants per hectare with a plant spacing of 15 cm and row spacing of 50 cm. Before laying the drip irrigation system and film mulching, each plot was fertilized with 525 $kg \cdot hm^{-2}$ of diammonium phosphate and 225 $kg \cdot hm^{-2}$ of potassium sulfate. The soybean seed was sown on 10 May and all treatments irrigated on 10 May with 90 mm of water to promote seedling emergence. The seedlings were thinned on 5 June, with two healthy seedlings per hole. Other management practices were consistent with standard local practice.

2.3 Measurements

2.3.1 Physiological parameters

Leaf gas exchange: four fully developed upper leaves from healthy plants in each treatment were randomly selected, and g_s, Tr and Pn measured using an LI-6400 portable photosynthesis system (LI-COR, Inc., Lincoln, NE, USA) between 10 and 11 am. Leaf instantaneous water use efficiency (WUE_{in}) was calculated as the ratio of Pn and Tr.

Stomatal aperture: the stomatal aperture was determined by a silicone rubber impression method[59]. A solution of collodion was applied in the same position on the abaxial side of soybean leaves, then after drying the leaves were torn slowly with transparent tape and stuck to a microscope slide. Three specimens were made from three randomly selected leaves from each treatment, and 10 microscopic fields were randomly selected to take pictures for each specimen using a biological microscope (Motic BA210, Motic China Group Co., Ltd, Xiamen, China) then enlarged 600 times. The number and width of stomata in each field were determined using the Motic Images Plus 2.0 software. The sampling was done on the same day as the measurements of leaf gas exchange.

Enzyme activity: eight holes on the two sides of the main vein of a soybean leaf were symmetrically taken using a punch with the inner diameter of 15 mm for measuring enzyme activity. Four replicate samples were taken for each treatment. The activities of SOD and POD were measured by the nitro blue tetrazolium reduction method and guaiacol method, respectively[60]. The samples were taken at 6 pm on the same day as the measurements of leaf gas exchange.

2.3.2 Soil water content

Two PVC access tubes were installed in the film-mulched soil of each plot. Volumetric soil water content was measured by Diviner 2000 system (Sentek Pty Ltd., Adelaide, Australia) at intervals of 0.1 m to a depth of 0.6 m, and calibrated using gravimetric soil water content. Measurements were made every 7–10 d, and additional measurements were made before and after each irrigation and rainfall event. The soil water content of W1 and W2 were 18.8%, 20.1%, 23.4% and 15.3%, 16.1%, 18.2%, respectively, when FZ was applied on 21 July, 8 August, 24 August.

2.3.3 Biomass

Biomass refers to total dry mass of leaves, stems and pods per plant at harvest. Three plants were randomly taken for each plot, and leaves, stems and pods were put into separate envelopes. These samples were first dried at 105°C for 30 min, and then dried at 80°C to constant mass.

2.3.4 Seed yield

Seed yield refers to dry seed mass at harvest. Two sampling areas (1 m × 1 m) were randomly selected from each plot and the average value was converted to dry seed mass per hectare of this plot. The seed yield for each treatment was calculated from the average of three replicates. WUE is the ratio of seed yield to total water consumption.

2.4 Data analysis

Simple statistical analysis and plotting were performed using Microsoft Excel 2010 (Microsoft, Redmond, WA, USA). Analysis of variance was performed using the generalized linear model procedure of SPSS 19.0 version software (IBM, Armonk, NY, USA). All the treatment means were compared for significant differences using Fisher's LSD test ($P \leqslant 0.05$).

3 Results and discussion

3.1 Effect of antitranspirant, FZ, on leaf superoxide dismutase and peroxidase activities under different irrigation rates

Figure 1 shows the variation in leaf SOD and POD activities by 1 d after spraying FZ during the pod bearing stage of soybean under different irrigation rates. Under W1, leaf SOD and POD activities were 318 $U \cdot g^{-1}$ and 592 $U \cdot g^{-1} \cdot min^{-1}$ for CK, and 440 $U \cdot g^{-1}$ and 788 $U \cdot g^{-1} \cdot min^{-1}$ for FZ, respectively. Compared with CK, FZ significantly improved the activities of SOD and POD by 38% and 33%, respectively. Similar results were found in previous studies of SA application on rice, tomato

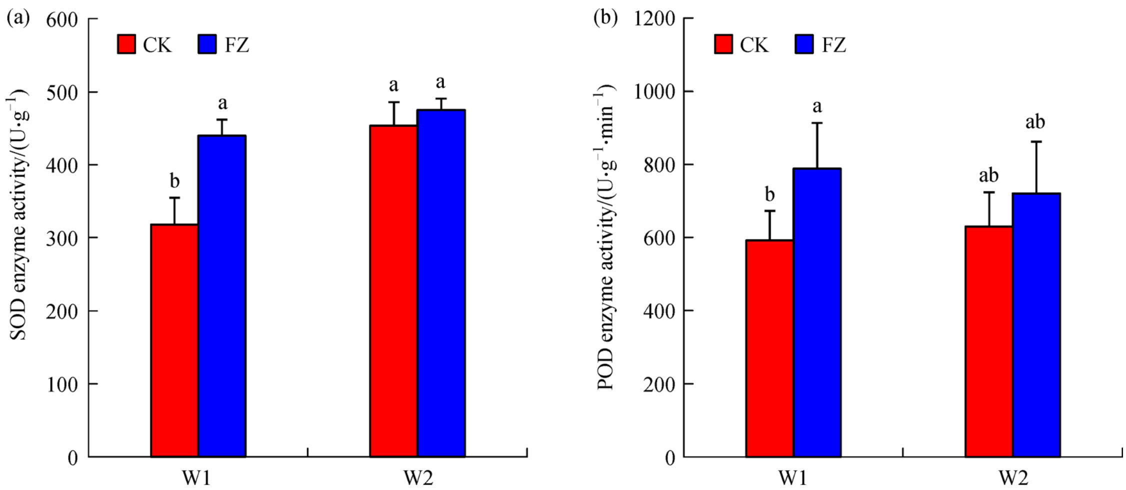

Fig. 1 Effect of foliar application of antitranspirant, FZ, during pod bearing stage on leaf superoxide dismutase (SOD) (a) and peroxidase (POD) (b) activities of soybean under different irrigation rates. W1, locally-used irrigation rate; W2, low irrigation rate; CK, water; different letters above the columns indicate significant differences at $P < 0.05$ level.

and wheat[4,6,8,29]. Under W2, FZ did not significantly affect leaf SOD and POD activities. This was probably because the soil water content was reduced to 40% of field capacity under W2, which led to severe water stress, and at this time soil water content became the main factor affecting leaf SOD and POD activities. Therefore, applying FZ only increased leaf SOD and POD activities significantly under W1.

3.2 Effect of antitranspirant FZ on leaf gas exchange under different irrigation rates

3.2.1 Effect of antitranspirant FZ at different growth stages on leaf gas exchange

Figure 2 shows the variation in leaf g_s, Tr, Pn and WUE_{in} by 1 d after spraying FZ during the pod bearing stage and the early and late pod filling stages of soybean. Under W1, there was no significant effect of FZ at different growth stages on leaf g_s, Pn and Tr, but FZ applied during the pod bearing stage significantly increased leaf WUE_{in} by 27% (Fig. 2d). Under W2, FZ applied at three growth stages reduced leaf g_s, Tr and Pn, but the reduction in Pn was less than that in Tr, which resulted in the increase of leaf WUE_{in}. For example, FZ applied during the pod bearing stage reduced leaf Tr and Pn by 37% and 22%, respectively, while it increased WUE_{in} by 25%. FZ applied during the early pod filling stage reduced g_s, Tr and Pn by 11%, 4% and 9%, respectively, but there was no significant effect on WUE_{in}. Therefore, leaf WUE_{in} under two irrigation rates increased significantly by 1 d after applying FZ during the pod bearing stage. This was probably because soybean needs more nutrients and water for vigorous growth during the pod bearing stage[61], and FZ contains N, P, K and other trace elements beneficial for soybean growth.

3.2.2 Effect of antitranspirant, FZ, during the pod bearing stage on leaf gas exchange at different times after application

Figure 3 shows the variations in leaf gas exchange at different times after applying FZ during the pod bearing stage (21 July). Under W1, applying FZ increased Pn, with this increase peaking by 7 d after spraying, and then gradually declining. This increase was probably because N, P, K and other trace elements in the FZ improved the activity of carboxylase per leaf area and promoted photosynthesis[62]. In addition, FZ significantly increased leaf POD activity under W1 (Fig. 1). Leaf POD can eliminate not only superoxide radicals, but also harmful H_2O_2 generated in the photosynthetic respiration process, which can improve plant photosynthesis[63]. FZ increased leaf g_s by 34% by 7 d after spraying, but did not significantly affect g_s by 10 and 13 d after spraying. Under W2, FZ significantly reduced g_s and Tr by 7 d after spraying, but did not significantly effect Pn and WUE_{in}, which was similar to the findings for applying kaolin to grapevines[9].

3.3 Effect of antitranspirant FZ on leaf stomatal aperture and frequency under different irrigation rates

Figure 4 shows the representative biomicroscope scanning images of the abaxial sides of soybean leaves by 1 d after applying FZ and CK under different irrigation rates. Figure 5 shows the effect of FZ application at the early pod filling stage on leaf stomatal aperture and frequency of soybean under different irrigation rates. The average stomatal aperture of FZ and CK leaves were 5.12 and

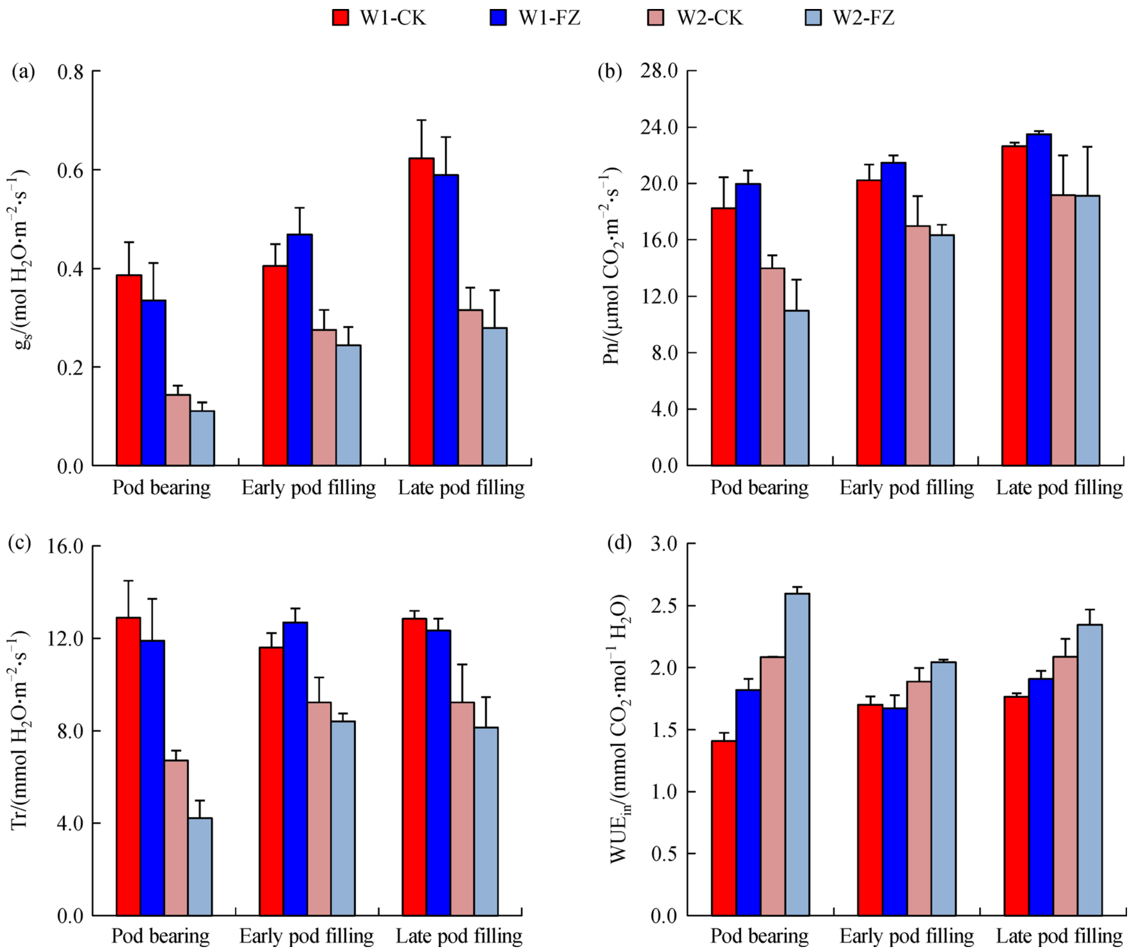

Fig. 2 Effect of foliar application of antitranspirant, FZ, at different growth stages on leaf stomatal conductance (g_s) (a), photosynthetic rate (Pn) (b), transpiration rate (Tr) (c), and instantaneous water use efficiency (WUE_{in}) (d) of soybean by 1 d after spraying. W1, locally-used irrigation rate; W2, low irrigation rate; CK, water.

4.79 μm, and the average stomatal frequencies were 497 and 507 n·mm^{-2}, respectively. FZ increased leaf stomatal aperture by 7% and reduced leaf stomatal frequency by 2% compared with CK. Under W2, the average stomatal aperture of FZ and CK leaves were 3.40 and 4.05 μm, and the average stomatal frequencies were 309 and 359 n·mm^{-2}, respectively. Compared with CK, FZ reduced the mean stomatal aperture and stomatal frequency by 16% and 14%, respectively. Figure 2a shows that under W2, FZ application during the early pod filling stage reduced g_s by partly closing stomata and reducing stomatal aperture, whereas under W1, FZ application during this stage increased g_s by enlarging stomatal opening. However, there was no significant effect of applying FZ on g_s under the two irrigation rates.

3.4 Effect of antitranspirant FZ on final biomass, seed yield and WUE of soybean under different irrigation rates

Final biomass, seed yield, harvest index, water consumption and WUE for all treatments are shown in Table 3. The effects of irrigation rate on final biomass, seed yield, total water consumption, harvest index, water consumption and WUE were all significant. But there were no significant effect of FZ on final biomass, seed yield, total water consumption, water consumption and WUE except harvest index. Compared with the CK, applying FZ three times under W1 from pod bearing stage to pod filling stage significantly increased the seed yield, harvest index and WUE by 23%, 11% and 22%, respectively, but there was no significant effect under W2. The increase of WUE after applying FZ improved seed yield but did not increase water consumption under W1. There were no significant effects under W2, which was also consistent with the effect of applying FZ on leaf g_s, Pn, Tr and antioxidant enzyme activities.

Under W1, the activities of SOD and POD increased after applying FZ during the pod bearing stage (Fig. 1), and promoted the photosynthesis (Fig. 2b; Fig. 3b), probably resulting in the increase in seed yield. Although leaf Tr temporarily increased by 7 d after spraying FZ, this did not affect the biomass accumulation. This was

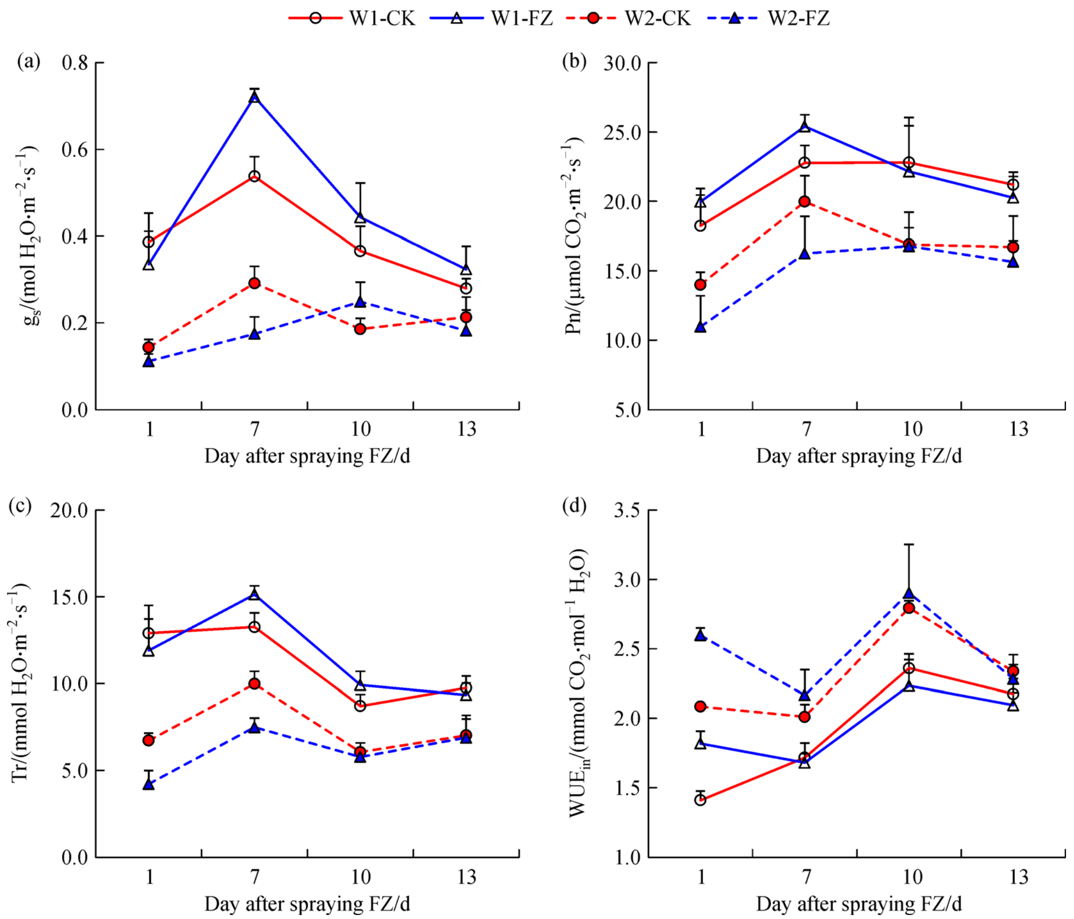

Fig. 3 Effect of foliar application of antitranspirant, FZ, at the pod bearing stage on leaf stomatal conductance (g_s) (a), photosynthetic rate (Pn) (b), transpiration rate (Tr) (c), and instantaneous water use efficiency (WUE_{in}) (d) of soybean at different times after spraying. W1, locally-used irrigation rate; W2, low irrigation rate; CK, water.

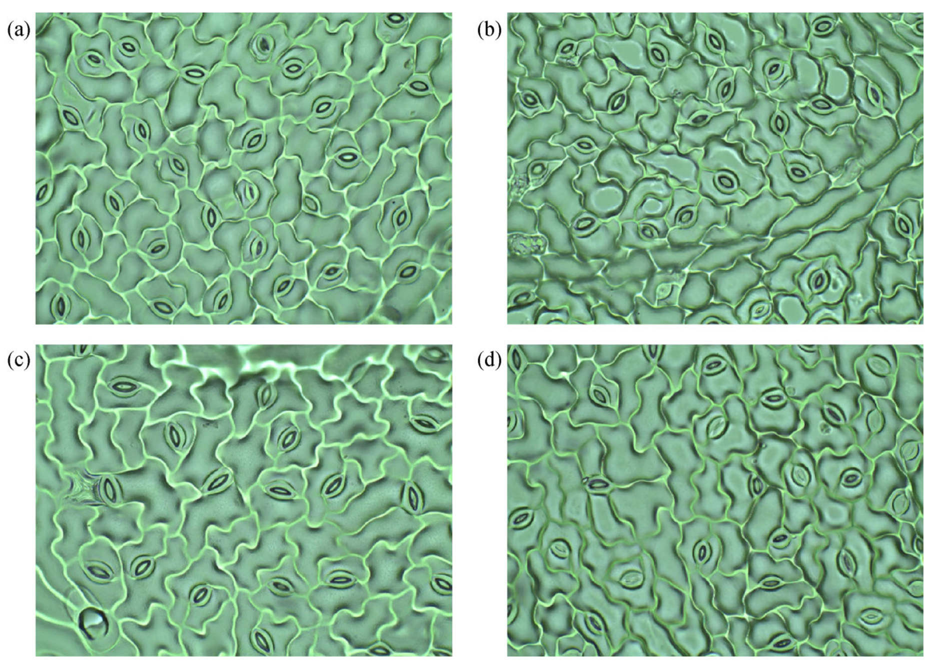

Fig. 4 Representative biomicroscope images of abaxial side of soybean leaves for different treatments. (a) W1-CK; (b) W1-FZ; (c) W2-CK; (d) W2-FZ. W1, locally-used irrigation rate; W2, low irrigation rate; CK, water; FZ, antitranspirant.

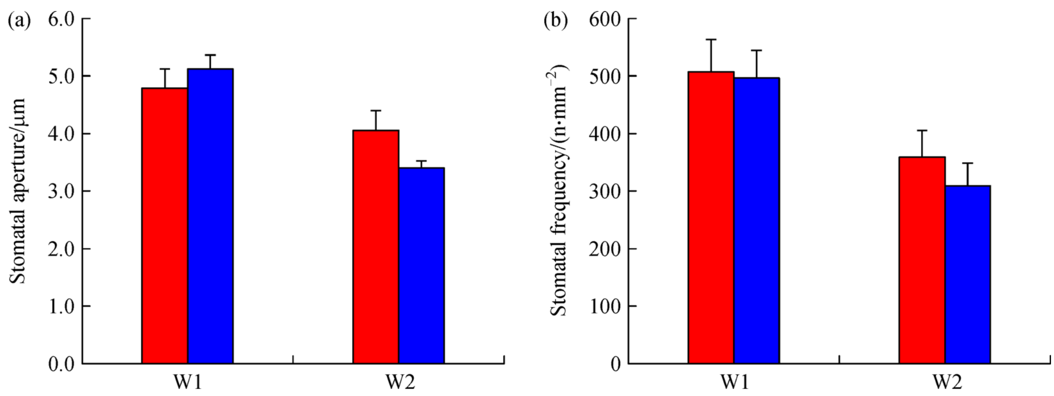

Fig. 5 Effect of foliar application of antitranspirant, FZ, at the early pod filling stage on leaf stomatal aperture (a) and frequency (b) for soybean under different irrigation rates. W1, locally-used irrigation rate; W2, low irrigation rate; CK, water.

Table 3 Effect of foliar application of antitranspirant, FZ, on final biomass, seed yield, harvest index, water consumption and water use efficiency (WUE) of soybean under different irrigation rates

Irrigation rate	Treatment	Final biomass per plant/g	Seed yield /($t \cdot hm^{-2}$)	Harvest index/%	Water consumption/mm	WUE /($kg \cdot m^{-3}$)
W1	CK	60.79±2.90 a	3.02±0.32 b	32.53±1.31 c	386.38±29.39 a	0.79±0.12 b
	FZ	67.09±12.57 a	3.70±0.09 a	36.13±0.04 b	384.92±0.43 a	0.96±0.02 a
W2	CK	48.42±5.67 b	2.69±0.26 b	39.05±1.60 a	287.74±11.04 b	0.93±0.07 ab
	FZ	48.34±9.74 b	2.59±0.11 b	39.85±1.12 a	298.82±14.38 b	0.87±0.07 ab
Significance test						
Irrigation rate		**	**	**	***	*
FZ		NS	NS	*	NS	NS
Irrigation rate *	FZ	NS	*	NS	NS	*

Note: W1, locally-used irrigation rate; W2, low irrigation rate; CK, water. Different letters in the same column indicate significant differences at $P < 0.05$ level. NS, no significance; ***, significance at $P < 0.001$; **, significance at $P < 0.01$; *, significance at $P < 0.05$.

probably the reason why total water consumption was not significantly affected. Thus WUE significantly increased under W1. However, under W2, physiological effects including the activities of SOD and POD, Pn, Tr, and stomatal frequency were affected, but applying FZ during the pod bearing stage temporarily decreased stomatal aperture and Tr by 7 d after spraying, so the final biomass, seed yield, total water consumption and WUE were not significantly affected. This was probably because severe water deficit affected the absorption of N, P, K and other trace elements in FZ under W2, leading to the lack of any significant effect on the growth of the soybean plants.

4 Conclusions

Under W1, applying FZ during the pod bearing stage increased the activities of SOD and peroxidase POD by 38% and 33%, respectively, but did not significantly affect leaf g_s, Tr, Pn, stomatal aperture and stomatal frequency. Seed yield and WUE increased by 23% and 22%, respectively, after applying FZ three times from pod bearing stage to pod filling stage.

Under W2, leaf g_s and Tr decreased significantly by 7 d after spraying FZ during the pod filling stage, but leaf Pn and WUE_{in} were not significantly affected. The stomatal frequency, stomatal aperture, g_s and Tr decreased by 16%, 14%, 26% and 14%, respectively, and WUE_{in} increased by 1 d after spraying FZ during the early pod filling stage. However, the final biomass, total water consumption, seed yield and WUE of soybean were not significantly affected by applying FZ three times from pod bearing stage to pod filling stage.

Although the application of the newly developed antitranspirant, FZ, did not have a significant positive effect on soybean under a low irrigation rate, the application of FZ can significantly improve seed yield and water use efficiency of soybean under normal irrigation rates. However, this study was for a single cultivar in one location, so further study of FZ on a wide range of cultivars under different climatic zones and irrigation conditions is needed before it is widely used in arid area.

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Compliance with ethics guidelines Shasha Ji, Ling Tong, Fusheng Li, Hongna Lu, Sien Li, Taisheng Du, and Youjie Wu declare that they have no conflict of interest or financial conflicts to disclose.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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