Influences of *Saccharomyces cerevisiae* on gas exchange and water-use efficiency in *Vicia faba* L.

Jing Gao1,2*, Nan Wang2, Gang Zhang1, Yonggang Yan1, Genxuan Wang3, Suiqi Zhang4

1College of Pharmacy and Shaanxi Qinling Application Development and Engineering Center of Chinese Herbal Medicine, Shaanxi University of Chinese Medicine, Xianyang 712046, China, 2Shaanxi Collaborative Innovation Center of Chinese Medicinal Resources Industrialization, Shaanxi University of Chinese Medicine, Xianyang 712083, China, 3College of Life Sciences, Zhejiang University, Hangzhou 310058, China 4Institute of Soil and Water Conservation, Northwest A&F University, Yangling 712100, China

*These authors contributed equally to this work.

**ABSTRACT**

To examine the hypothesis that microbes can function as antitranspirants regulating stomatal behavior, we studied the effect of *Saccharomyces cerevisiae* strain BY4741 on gas exchange and water-use efficiency (WUE) in *Vicia faba* L. Stomatal aperture and photosynthetic parameters were analyzed every day for 7 days after treatment. In the present study, spraying yeast decreased the stomatal aperture. On the 4th, 5th, 6th, and 7th days after the foliar application of yeast, a decreased in net photosynthetic rate ($P_n$) was observed. This decrease was accompanied with decreases in stomatal conductance ($g_s$), intercellular CO$_2$ concentration ($C_i$), and transpiration ($T_r$). The carboxylation efficiency (CE) was reduced by yeast on the 1st day after treatment. On the 1st, 2nd, and 3rd days of treatment, the yeast dramatically decreased the maximum photochemical efficiency of photosystem (PS) II ($F_v/F_m$) and increased non-photochemical quenching (NPQ); however, these parameters eventually recovered to their normal levels. The marked decrease of $q_P$ was observed on the 1st and 2nd days after yeast treatment. At the beginning of the treatment, the yeast decreased electron transport rate (ETR), but later increased it. Both concentrations of yeast ($1 \times 10^8$ and $1 \times 10^9$ CFU mL$^{-1}$) increased the WUE on the 4th, 5th, 6th, and 7th days of treatments. At the concentrations of $1 \times 10^8$ CFU mL$^{-1}$, yeast acted as a more effective antitranspirant, showing higher WUE, than at the concentration of $1 \times 10^9$ CFU mL$^{-1}$. $P_n$ was positively correlated with $T_r$ and $g_s$ on the 7th day of the treatment. Moreover, $T_r$ was much more sensitive to yeast than was $P_n$. In general, the foliar application of yeast resulted in decreased $T_r$ and increased WUE. The yeast-induced decrease in photosynthesis is mainly caused by stomatal closure, suggesting that yeast can be used as an antitranspirant or a priming agent for improving drought tolerance in plants.

**Keywords:** Bio-control; Gas exchange; *Vicia faba*; Water-use efficiency (WUE); Yeast

**INTRODUCTION**

Owing to the severe shortage of water resources, improving water-use efficiency (WUE) has received considerable attention in plant physiology. The methods for improving WUE in agriculture include: (1) irrigation techniques such as sprinkler irrigation, drip irrigation, micro-irrigation, regulated deficit irrigation, etc. (Fereres and Soriano, 2016); (2) cultivation practices such as mulching, fertilizer management, etc. (Yunusa et al., 1994; Hussain and Al-Jaloud, 1995); (3) water-saving chemical treatment using phenylmercury acetate, atrazine, alachlor, triazolone, etc. (Squire and Jones, 1971); (4) gene-editing (Sivamani et al., 2000; Melotto et al., 2006). However, the techniques mentioned above are uneconomical and pollute the environment.

Among the alternative and eco-friendly methods, plant growth-promoting microorganisms (PGPMs) play an important role in the sustainable agriculture industry (Brilli et al., 2019). PGPMs can enhance crop production through a number of direct and indirect mechanisms, such as by promoting the uptake of water and nutrients (Abhilash et al., 2016), by inhibiting pathogen invasion, and by affecting the production of growth regulators. Previous studies have focused on the interactions between agricultural plants and microorganisms in the soil or rhizosphere. More and more studies have proved that phyllosphere microorganisms also play important roles in plant growth (Batool et al., 2016; Stone et al., 2018).

A vast number of microorganisms colonize the leaf surface of terrestrial plants and occur in areas, such as stomatal
opening, that foster closer interactions with the host plant (Li et al., 2019). Some phyllosphere microorganisms can produce abscisic acid (ABA) and control stomatal movement (Stone et al., 2018). Stomata are the pores on the leaf surface formed by pairs of epidermal guard cells, which are important portals for controlling gas and water exchange in plants (Melotto et al., 2006; Franks et al., 2015; Franks and Farquhar, 2015). Thus, stomata hold the key to increase plant WUE (Davies et al., 2002). A recent study showed that partial stomatal closure can improve WUE (Li et al., 2014). The exogenous application of an antitranspirant can induce stomatal closure, resulting in increased WUE. Identifying natural and safe antitranspirants is becoming a high research priority (Davies et al., 2002). Moreover, stomatal movement can be influenced by phyllosphere microorganisms (Li et al., 2014). However, it is still not clear whether phyllosphere microorganisms can function as antitranspirants to enhance WUE.

Epiphytic yeast (Saccharomyces cerevisiae), a phyllosphere fungus, is a natural plant growth promoter and is used as a safe chemical fertilizer in agriculture (Shalaby and El-Nady, 2008; Ziedan and Farrag, 2011). The cell wall of S. cerevisiae consists of a number of polymers including chitosan (2%) (Brady et al., 1994), which has been used to enhance growth, stimulate immune system, and conserve water use in plants (Uthairatanakij et al., 2007; Bistgani et al., 2017). Thus, yeast is perhaps considered as an appropriate antitranspirant to regulate stomatal behavior and improve WUE. Therefore, the objectives of this study were to: 1) determine whether epiphytic yeast influences stomatal movement; 2) explore the effect of exogenous spraying of yeast on photosynthesis, and 3) determine the appropriate concentration of yeast for spraying to improve WUE.

MATERIALS AND METHODS

Chemicals and Reagents

Yeast Extract, peptone, glucose, 2-(N-morpholino)ethanesulfonic acid (MES), and KCl were purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). Broad bean seeds were purchased from Shanghai Nongle Seed Company (Shanghai, China). Vermiculite, perlite, and nutritional soil were purchased from the local market of Hangzhou, China.

Instrumentation

DSZ5000X microscope (UOP, Chongqing, China) fitted with a Canon Powershot G10 camera has a clear microscopic observation and imaging system. The portable photosynthetic open-system (CI-340, 4845NW Camas Meadows Drive, Camas, WA, 98607, USA) equipped with data memory, a CO₂/H₂O gas analyzer, and a flow control system provides automatic control independent of the environmental conditions of the interior of the leaf chamber. The MAXI version of the IMAGING-PAM M-Series chlorophyll fluorescence system (Heinz-Walz GmbH, Effeltrich, Germany) is composed of a light emitting diode (LED) power supply unit, an LED light source, a charge-coupled device (CCD), data line, software, etc. Chlorophyll fluorescence imaging provides a powerful, non-invasive tool for investigating leaf photosynthesis under natural conditions.

Plant materials

In the present study, Vicia faba L. ‘Daqingpi’ was used as the plant material. It was grown in an open chamber of Zhejiang University at Hangzhou (120°2’E, 30°3’N), Zhejiang province, located along the southeast coast of China with subtropical monsoon climate for 5 weeks. Seeds were soaked in water for 4 days and then sown in plastic pots (12 cm diameter × 14.5 cm height) in April, filled with vermiculite, perlite, and nutritional soil (1:1:1, by vol.). The plants were irrigated daily and grown under natural irradiance conditions in the open chamber.

Yeast cultivation

Saccharomyces cerevisiae strain BY4741 was grown in YPD liquid medium (1% yeast extract, 2% peptone, and 2% glucose) at 30°C for 48 h. After 10 min of centrifugation at 3000 r min⁻¹, the supernatant was discarded, and the harvested cells were resuspended at the final concentrations of 1 × 10⁶ and 1 × 10¹⁰CFU mL⁻¹ in ultrapure water without any detergents.

Stomatal aperture measurement

Five leaf disks (5 mm in diameter) per treatment were randomly collected from different plants. Freshly prepared abaxial epidermal strips were peeled carefully from the abaxial surface of the leaves and immediately put into MES/KCl buffer (10 mM MES, 50 mM KCl, pH 6.15). The epidermal strips were transferred on a glass slide for microscopic analysis. The stomatal apertures were determined by digital images captured using a DSZ5000X microscope. The width of the stomatal aperture was measured using the software Image Pro Plus 6.0 software (Media Cybernetics, Silver Springs, MD).

To avoid any potential rhythmic effects on stomatal aperture, the experiments were always started at the same time every day for 7 days. The data presented are the means of 90 stomatal apertures ± SE.

Gas exchange measurements

Five-week-old plants were sprayed with two different concentrations of yeast, and the controls were sprayed with water. Both adaxial and abaxial surfaces of all expanded leaves were sprayed. The leaf gas exchange parameters (net photosynthetic rate, Pn; transpiration rate, Tr; intercellular CO₂ concentration, Ci; stomatal conductance, gs) were measured using a portable photosynthetic open-system.
(CI-340, 4845NW Camas Meadows Drive, Camas, WA, 98607, USA) daily for 7 days following the yeast spray treatments. Before measurements, the plants were allowed to acclimate to sufficient light irradiance for more than 1 h. During measurements, a leaf-chamber light intensity of 800 µmol m⁻² s⁻¹ was supplied by an LED source on the adaxial leaf surface, the leaf temperature was 25°C, and the ambient CO₂ concentration was 400 µmol mol⁻¹ (Li et al., 2014). For each measurement, two upper, fully expanded, terminal, and well-exposed leaves on one plant per plot were chosen. The yeast-treated leaves were chosen from the plants with the most uniform coating. All leaf gas-exchange measurements were performed between 10:00 and 14:00, using eight leaves randomly collected from four plants. Data were recorded after 3–4 min, when the steady-state photosynthesis was achieved. WUE was calculated as $P_n / T_\text{r}$ and carboxylation efficiency (CE) as $P_n/\text{C}_i$. The relationship between $P_n$ and $g$ was evaluated by a logarithmic function and that between $T_\text{r}$ and $g$ was evaluated by a linear function. The correlation and regression analyses were conducted using SPSS version 13.0 (SPSS, Chicago, USA).

**Chlorophyll fluorescence measurements**
Leaves used for chlorophyll fluorescence assay were collected 7 days after treatment. The maximum photochemical efficiency of PS II ($F_{\text{V}}/F_{\text{m}}$), electron transport rate (ETR), non-photochemical quenching (NPQ), and photochemical quenching ($q_P$) were analyzed with the MAXI version of the IMAGING-PAM M-Series chlorophyll fluorescence system. Plants were dark-adapted for 30 min prior to measurements. The details can be obtained from Kościelniak and Biesaga-Kościelniak (2006). Saturation pulses were given every 20 s (Gao et al., 2013). This experiment was repeated eight times (n=8).

**Statistical analyses**
Standard error (SE) was calculated for each treatment. Differences among treatments were compared using Tukey’s HSD (honestly significant difference) test. A P-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using the SPSS 13.0 package.

**RESULTS**

**Stomatal closure by yeast**
Foliar spraying with yeast induced stomatal closure (Fig. 1). During 7 days after spraying, both concentrations of yeast ($1 \times 10^6$ and $1 \times 10^8$ CFU mL⁻¹) significantly decreased the stomatal aperture (Fig. 1).

**Effects of yeast on leaf gas-exchange**
The $g$ and $T_\text{r}$ values were almost lower in the yeast-treated plants than in the control plants during 7 days after treatment (Fig. 2B, C). On the 4th, 5th, 6th and 7th days, both $P_n$ and $C_i$ were significantly lower in the yeast-treated plants than in the controls (Fig. 2A, D). On the 2nd, 3rd, 4th, 5th, 6th, and 7th days, WUE was higher in the plants treated with both concentrations ($1 \times 10^6$ and $1 \times 10^8$ CFU mL⁻¹) of yeast than in the controls (Fig. 2E). Under yeast treatment, CE was decreased only on the 1st day (Fig. 2F).

**Effects of yeast on chlorophyll fluorescence**
The yeast treatment induced a significantly lower $F_{\text{V}}/F_{\text{m}}$ ratio and higher NPQ than the control treatment on the 1st, 2nd, and 3rd days (Fig. 3A, B). However, no significant differences in $F_{\text{V}}/F_{\text{m}}$ and NPQ were observed between the yeast-treated and control plants (Fig. 3A, B). $q_P$ was decreased by yeast on the 1st and 2nd days after treatment (Fig. 3D). The ETR was significantly decreased on the 1st day and then increased on the 3rd and 4th days after the yeast treatment (Fig. 3C).

**Relationships between $g$ and $P_n$, $T_\text{r}$, and WUE on the 7th day**
The relationship between $P_n$ and $g$ was well represented by a logarithmic function ($r^2 = 0.93$) (Fig. 4A). Fig. 4B shows a linear relationship between $T_\text{r}$ and $g$ ($r^2 = 0.99$) on the 7th day after spraying. Fig. 4C shows a relationship between WUE and $g$ on the 7th day after spraying, wherein WUE increased linearly with decreasing $g$ and reached a peak at approximately 10 mmol mol⁻¹ when $g$ approached 20 mmol m⁻² s⁻¹; however, when $g$ decreased further, WUE decreased dramatically and reached close to zero.

**Interrelationships between gas-exchange parameters on the 7th day**
As shown in Fig. 5, $P_n$ was more correlated with $T_\text{r}$ and $g$ and WUE with $C_i$ than with other gas-exchange parameters. In addition, a negative correlation was observed between $C_i$ and other gas-exchange parameters.

**DISCUSSION**
Stomata can sense environmental changes and then regulate transpiration and gas exchange accordingly (Franks et al., 2015; Jones 1998). The interactions between microbes and plant compounds play important roles in microorganism-induced stomatal behavior (Ortíz-Castro et al., 2009). Yeast, a non-pathogenic fungus, can induce stomatal closure, but not cell death at the epidermal level (Khokon et al., 2010). The yeast ascospore wall consists of chitosan (Brady et al., 1994), which can induce elevations in the concentration of free cytosolic calcium ($[\text{Ca}^{2+}]_{\text{cyt}}$) in guard cells (Klusener et al., 2002; Khokon et al., 2010) and lead to stomatal closure. The similar responses occurred in the present study following the application of living yeast cells. Our results indicated that the spraying of yeast cells led to the
Fig 1. Changes in stomatal aperture of *Vicia faba* after treatment with two concentrations of yeast for 7 days. Error bars indicate ± SE (n=90). The different letters represent significant differences (P<0.05) and ns represents no significant difference (P>0.05).

Fig 2. Changes in net photosynthetic rate, \( P_n \) (A), stomatal conductance, \( g_s \) (B), transpiration rate, \( T_r \) (C), intercellular \( \text{CO}_2 \) concentration, \( C_i \) (D), water use efficiency (WUE; \( P_n/T_r \)) (E) and Carboxylation efficiency (CE; \( P_n/C_i \)) (F) of *Vicia faba* after treatment with two concentrations of yeast for 7 days. Error bars indicate ± SE (n=8). The different letters represent significant differences (P<0.05) and ns represents no significant difference (P>0.05).

Closure of stomatal aperture accompanied by a reduction in \( g_s \) at the whole-plant level (Fig. 2A).

Leaf gas exchange and chlorophyll fluorescence are considered as powerful and efficient parameters in plant ecophysiology studies (Maxwell and Johnson, 2000). In addition, they are non-intrusive indicators for rapid assessment of *in vivo* photosynthesis in biotic and abiotic stress responses of plants (Maxwell and Johnson, 2000; Sui 2015). \( P_n \) was significantly lower in the yeast-treated plants than in the control plants on the 4th, 5th, 6th and 7th days (Fig. 2A). \( g_s \) and \( C_i \) are important parameters to assess limitations to photosynthesis (Jiang et al., 2006); they were both decreased on the 4th, 5th, 6th and 7th days after treatment with yeast (Fig. 2B, C). In addition, the results showed that \( P_n \) was more positively related to \( g_s \) than to \( C_i \) on the 7th day (Fig. 5). Therefore, the serious depression in \( P_n \) after the yeast treatment was due to the closure of...
stomata as shown by the decrease in $g_s$ (Fig. 2B), which, in turn, contributed to reduced $T_r$ (Fig. 2C).

Similar conclusions could be drawn from the changes in CE and chlorophyll fluorescence parameters. The marked reduction in CE was observed only on the 1st day after the yeast treatment (Fig. 2F). CE indicates carbon assimilation (Ding et al., 2014), and its decrease under yeast treatment was probably due to the reduction in activity and/or quantity of Rubisco (Zhang et al., 2010). $P_n$ decreased after 6 days of yeast treatment, while CE showed no decrease, indicating that yeast did not impair mesophyll processes (Zhang et al., 2010). Therefore, the decrease in $P_n$ was caused by stomatal factors under yeast treatment.

In our research, the reduction in $P_n$ was a side effect of the antitranspirant treatment (Fig. 2A). A previous study showed that the application of an antitranspirant restricted $P_n$ and decreased plant yield (Kettlewell et al., 2010). In contrast, kaolin, a film antitranspirant, reduced $P_n$, but provided a notable yield increase (Cantore et al., 2009). A recent study also showed that yeast had no effect on $P_n$, but increased grain yield under drought conditions (Gao et al., 2014). These inconsistent results might be due to the differences in plant variety, development stages, and environment conditions. In addition, it should be noted that $P_n$ was defined at a leaf scale in the present study, and plant behavior can be different at the level of individual leaves and of the whole canopies (Munekage et al., 2004; Franks et al., 2015). Despite causing a reduction in photosynthesis, an antitranspirant can increase the growth of stem internodes through cell expansion (Héroult et al., 2013). Based on its richness in cytokinins and other growth-regulating substances, yeast is suggested to play a beneficial role in cell division and cell enlargement (Shalaby and El-Nady, 2008). In the future, it would be interesting to study the methods of applying yeast to enhance yield under elevated WUE conditions.

The $F_v/F_m$ ratio reflects the maximal photochemical yield of PS II center (Maxwell and Johnson, 2000; Sui, 2015).
Although the $F_v/F_m$ ratio was dramatically decreased on the 1st, 2nd, and 3rd days of the yeast treatment, it was eventually recovered to its normal level (Fig. 3B), indicating that the function of PS II was not significantly damaged by yeast. NPQ is a feedback regulatory mechanism induced upon exposure to a photon flux density in excess of what can be used with the maximum quantum yield of PS II, which is linearly related to heat dissipation (Maxwell and Johnson, 2000). On the 1st, 2nd, and 3rd days of the yeast treatment, there was an increase in NPQ (Fig. 3A, C), which denoted an increase in energy dissipation through non-photochemical processes. qP is another widely used fluorescence parameter, representing the openness of the PS II reaction centers and redox state of the primary quinone acceptor of PS II (QA) (Maxwell and Johnson, 2000). On the 1st and 2nd days, a decrease in qP might indicate that the yeast treatment induced the closure of the PS II reaction center. ETR is indicative of the relative amount of electrons passing through PS II during steady-state photosynthesis (Franks et al., 2015). In the present study, the high ETR accompanied by low $P_n$, low $g_s$, and reduced stomatal aperture indicated that a decrease in $P_n$ was caused by stomatal limitation.

Stomata allow CO$_2$ to enter the leaf and provide a pathway for water vapor diffusion out of the leaf to the atmosphere (Melotto et al., 2006; Franks and Farquhar, 2007; Héroult et al., 2013; Franks et al., 2015). In order for plants to use water efficiently, stomata must ensure an appropriate balance between CO$_2$ demands for photosynthesis and water loss through transpiration (Héroult et al., 2013). WUE increased with a decrease in $g_s$ and reached a peak at $g_s \approx 20$ mmol m$^{-2}$ s$^{-1}$, and then declined dramatically as the $g_s$ continued to decline (Fig. 4C). The optimal stomatal behavior, which reflects a trade-off between water conservation and high CO$_2$ assimilation, could allow constant $P_n/T_r$ with changing environment (Héroult et al., 2013; Manzoni et al., 2013). Additionally, the optimum stomatal aperture could be between the fully open aperture and that giving maximum WUE (Bazzaz et al., 1974). The positive linear correlation between $T_r$ and $g_s$ (Fig. 4B) indicated that the stomatal resistance contributes more to the total leaf resistance for H$_2$O diffusion than for CO$_2$ uptake (Franks et al., 2015). The data obtained from this study provided evidence that yeast application caused more decline in transpiration than in photosynthesis, thus leading to an increase in WUE. Similar results were reported by Nilsen and Orcutte (Nilsen and Orcutte, 1996). ABA induced higher WUE because it induced stomatal closure, and the reduction in $P_n$ was much lower than that in $T_r$. The data showed that $1 \times 10^{10}$ CFU mL$^{-1}$ induced more stomatal resistance than $1 \times 10^{6}$ CFU mL$^{-1}$ of yeast, while the maximum WUE occurred at $1 \times 10^{6}$ CFU mL$^{-1}$ of yeast. Therefore, as an antitranspirant, yeast was more effective at the concentration of $1 \times 10^{6}$ CFU mL$^{-1}$ than at $1 \times 10^{10}$ CFU mL$^{-1}$. Thus, in order to achieve the maximum WUE, it is important to have optimum stomatal aperture.
CONCLUSION

The results showed that the foliar application of yeast significantly increased the WUE of *V. faba* plants by regulating the stomatal movement. The yeast-induced decreases in photosynthesis are mainly due to stomatal limitation rather than the direct effect on the capacity of the photosynthetic apparatus. It could be an effective antitranspirant with its low environmental impact and economic cost.

ACKNOWLEDGEMENT

This research was supported by the National Science Foundation for Young Scientists of China (Grant No.31600320), and Subject Innovation Team of Quality Control and Resources Development of “Qin medicine” of Shaanxi University of Chinese Medicine (2019-QN01). We thank Shaanxi Qinling Application Development and Engineering Center of Chinese Herbal Medicine for providing the experimental platform.

Authors’ contributions

Nan Wang designed the study; Jing Gao carried out the project and drafted the manuscript; Yonggang Yan and Gang Zhang participated in data analysis; Genxuan Wang and Suiqi Zhang edited the article. All authors read and approved the final manuscript.

REFERENCES

Abhilash, P. C., R. K. Dubey, V. Tripathi, V. K. Gupta and H. B. Singh. 2016. Plant growth-promoting microorganisms for environmental sustainability. Trends Biotechnol. 34 847-850.

Batool, F., Y. Rehman and S. Hasnain. 2016. Phytoplane associated plant bacteria of commercially superior wheat varieties exhibit superior plant growth promoting abilities. Front. Life Sci. 9313-322.

Bazzaz, F. A., G. L. Rolfe and R. W. Carlson. 1974. Effect of Cd on photosynthesis and transpiration of excised leaves of corn and sunflower. Physiol. Plantarum. 32 373-376.

Bistgani, Z. E., S. A. Siadat, A. Bakhshandeh, A. G. Pirbalouti and M. Hashemi. 2017. Interactive effects of drought stress and chitosan application on physiological characteristics and essential oil yield of *Thymus daenensis* Celak. Crop J. 5 407-415.

Brady, D., A. Stoll, L. Starke and J. Duncan. 1994. Chemical and enzymatic extraction of heavy metal binding polymers from isolated cell walls of *Saccharomyces cerevisiae*. Biotechnol. Bioeng. 44 297-302.

Brilli, F., S. Pollastri, A. Raio, R. Baraldi, L. Neri, P. Bartolini, A. Podda, F. Loreto, B. E. Maserti and R. Ballestrini. 2019. Root colonization by *Pseudomonas chlororaphis* primes tomato (*Lycopersicum esculentum*) plants for enhanced tolerance to water stress. J. Plant Physiol. 232 82-93.

Cantore, V., B. Pace and R. Albrizio. 2009. Kaolin-based particle film technology affects tomato physiology, yield and quality. Environ. Exp. Bot. 66 279-288.

Davies, W. J., S. Wilkinson and B. Loveys. 2002. Stomatal control by chemical signalling and the exploitation of this mechanism to increase water use efficiency in agriculture. New Phytol. 153 449-460.

Ding, Z., T. Li, X. Zhu, X. Sun, S. Huang, B. Zhou and M. Zhao. 2014. Three photosynthetic patterns characterized by cluster analysis of gas exchange data in two rice populations. Crop J. 2 22-27.

Fereres, E. and M. A. Soriano. 2006. Deficit irrigation for reducing agricultural water use. J. Exp. Bot. 58 147-159.

Franks, P. J. and G. D. Farquhar. 2007. The mechanical diversity of stomata and its significance in gas-exchange control. Plant Physiol. 143 78-87.

Franks, P. J., T. W Doheny-Adams, Z. J. Britton-Harper and J. E. Gray. 2015. Increasing water-use efficiency directly through genetic manipulation of stomatal density. New Phytol. 207 188-195.

Gao, J., N. Wang, S. S. Xu, Y. Li, Y. Wang and G. X. Wang. 2013. Exogenous application of trehalose induced H$_2$O$_2$ production and stomatal closure in *Vicia faba*. Biol. Plantarum. 57 380-384.

Gao, J., N. Wang, Y. Li, Y. Wang and G. X. Wang. 2014. Influence of *Saccharomyces cerevisiae* on gas exchange and yield attributes in rice under drought conditions. Biol. Agric. Hortic. 30 52-61.

Héroult, A., Y. S. Lin, A. Bourne, B. E. Medlyn and D. S. Ellsworth. 2013. Optimal stomatal conductance in relation to photosynthesis in climatically contrasting *Eucalyptus* species under drought. Plant Cell Environ. 36 262-274.

Hussain, G. and A. A. Al-Jaloud. 1995. Effect of irrigation and nitrogen on water use efficiency of wheat in Saudi Arabia. Agric. Water Manage. 27 143-153.

Jiang, Q., D. Roche, T. A. Monaco and D. Hole. 2006. Stomatal conductance is a key parameter to assess limitations to photosynthesis and growth potential in barley genotypes. Plant Biol. 8 515-521.

Jones, H. G. 1998. Stomatal control of photosynthesis and transpiration. J. Exp. Bot. 49 387-398.

Kettlewell, P. S., W. L. Heath and I. M. Haigh. 2010. Yield enhancement of droughted wheat by film antitranspirant application Rationale and evidence. Agric. Sci. 1 143-147.

Khokon, M. A. R., M. A. Hossain, S. Munemasa, M. Uraji, Y. Nakamura, I. C. Moril Y. Murata. 2010. Yeast elicitor-induced stomatal closure and peroxide-mediated ROS production in Arabidopsis. Plant Cell Physiol. 51 1915-1921.

Klüsener, B., J. J. Young, Y. Murata, G. J. Allen, I. C. Moril V. Hugouvieux and J. I. Schroeder. 2002. Convergence of calcium signaling pathways of pathogenic effectors and absicic acid in Arabidopsis guard cells. Plant physiol. 130 2152-2163.

Kościelniak, J. and J. Biesaga-Kościelniak. 2006. Photosynthesis and non-photochemical excitation quenching components of chlorophyll excitation in maize and field bean during chilling at different photon flux density. Photosynthetica. 44 174-180.

Li, Y., S. S. Xu, J. Gao, S. Pan and G. X. Wang. 2014. Chlorella induces stomatal closure via NADPH oxidase-dependent ROS production and its effects on instantaneous water use efficiency in *Vicia faba*. PLoS One. 9 e93290.

Li, Y., S. S. Xu, J. Gao, S. Pan and G. X. Wang. 2014. Chlorella induces stomatal closure via NADPH oxidase-dependent ROS production and its effects on instantaneous water use efficiency in *Vicia faba*. PLoS One. 9 e93290.

Lin, W., X. Z. Liu, S. S. Zhou and C.F. Liu. 2019. Influence of plastic film mulch on maize water use efficiency in the Loess Plateau of China. Agric. Water Manage. 224 105710.
Manzoni, S., G. Vico, S. Palmroth, A. Porporato and G. Katul. 2013. Optimization of stomatal conductance for maximum carbon gain under dynamic soil moisture. Adv. Water Resour. 62 90-105.

Maxwell, K. and G. N. Johnson. 2000. Chlorophyll fluorescence a practical guide. J. Exp. Bot. 51 659-668.

Melotto, M., W. Underwood, J. Koczan, K. Nomura and S. Y. He. 2006. Plant stomata function in innate immunity against bacterial invasion. Cell. 126 969-980.

Munekage, Y., M. Hashimoto, C. Miyake, K. I. Tomizawa, T. Endo, M. Tasaka and T. Shikanai. 2004. Cyclic electron flow around photosystem I is essential for photosynthesis. Nature. 429 579-582.

Nilsen, E. T. and D. M. Orcutte. 1996. Phytohormones and plant responses to stress. In E. T. Nilsen and D. M. Orcutte (Ed.), Physiology of Plant under Stress Abiotic Factors, John Wiley and Sons, New York, pp. 183-198.

Ortiz-Castro, R., H. A. Contreras-Cornejo, L. Macías-Rodríguez and J. López-Bucio. 2009. The role of microbial signals in plant growth and development. Plant Signal. Behav. 4 701-712.

Shalaby, M. and M. F. El-Nady. 2008. Application of Saccharomyces cerevisiae as a biocontrol agent against Fusarium infection of sugar beet plants. Acta Biol. Szegediensis. 52 271-275.

Sivamani, E., A. Bahieldin, J. M. Wraith, T. Al-Niemi, W. E. Dyer, T. H. D. Ho and R. Qu. 2000. Improved biomass productivity and water use efficiency under water deficit conditions in transgenic wheat constitutively expressing the barley HVA1 gene. Plant Sci. 155 1-9.

Squire, G. R. and M. B. Jones. 1971. Studies on the mechanisms of action of the antitranspirant phenylmercuric acetate, and its penetration into the mesophyll. J. Exp. Bot. 22 980-991.

Stone, B. W. G., E. A. Weingarten and C. R. Jackson, 2018. The role of the phyllosphere microbiome in plant health and function. Annu. Plant Rev. Online. 1 1-24.

Sui, N. 2015. Photoinhibition of Suaeda salsa to chilling stress is related to energy dissipation and water-water cycle. Photosynthetica. 53 207-212.

Uthairatanakij, A., J. T. da Silva and K. Obsuwan. 2007. Chitosan for improving orchid production and quality. Science. 1 1-5.

Yunusa, I. A. M., R. H. Sedgley and K. M. H. Siddique. 1994. Influence of mulching on the pattern of growth and water use by spring wheat and moisture storage on a fine textured soil. Plant Soil. 160 119-130.

Zhang, L., H. Xu, J. C. Yang, W. D. Li, G. M. Jiang and Y. G. Li. 2010. Photosynthetic characteristics of diploid honeysuckle (Lonicera japonica Thunb.) and its autotetraploid cultivar subjected to elevated ozone exposure. Photosynthetica. 48 87-95.

Ziedan, E. and E. S. Farrag. 2011. Application of yeasts as biocontrol agents for controlling foliar diseases on sugar beet plants. J. Agric. Tech. 7 1789-1799.