Emirates Journal of Food and Agriculture. 2019. 31(12): 902-909
doi: 10.9755/ejfa.2019.v31.i12.2041
http://www.ejfa.me/

RESEARCH ARTICLE

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

Jing Gao[1a]*, Nan Wang[2], Gang Zhang[1], Yonggang Yan[1], Genxuan Wang[3], Suiqi Zhang[4]

[1]College of Pharmacy and Shaanxi Qinling Application Development and Engineering Center of Chinese Herbal Medicine, Shaanxi University of Chinese Medicine, Xianyang 712046, China, [2]Shaanxi Collaborative Innovation Center of Chinese Medicinal Resources Industrialization, Shaanxi University of Chinese Medicine, Xianyang 712083, China, [3]College of Life Sciences, Zhejiang University, Hangzhou 310058, China [4]Institute of Soil and Water Conservation, Northwest A&F University, Yangling 712100, China
[a]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 qP was observed on the 1st and 2nd days after yeast treatment. At the beginning of the treatment, the yeast decreased electron the transport rate (ETR), but later increased it. Both concentrations of yeast (1×10^6 and 1×10^{10} CFU mL^{-1}) increased the WUE on the 4th, 5th, 6th, and 7th days of treatments. At the concentrations of 1×10^6 CFU mL^{-1}, yeast acted as a more effective antitranspirant, showing higher WUE, than at the concentration of 1×10^{10} 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

***Corresponding author:**
Jing Gao, College of Pharmacy and Shaanxi Qinling Application Development and Engineering Center of Chinese Herbal Medicine, Shaanxi University of Chinese Medicine, Xianyang 712046, China. **E-mail:** gaojing@sntcm.edu.cn

Received: 23 March 2019; **Accepted:** 12 November 2019

opening, that foster closer interactions with the host plant (Lin 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 been 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 CO_2/H_2O 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 1×10^6 and 1×10^{10} CFU 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, P_n; transpiration rate, T_r; intercellular CO_2 concentration, C_i; stomatal conductance, g_s) 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 CO_2 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_r and carboxylation efficiency (CE) as P_n/C_i. The relationship between P_n and g_s was evaluated by a logarithmic function and that between T_r and g_s 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_v/F_m), electron transport rate (ETR), non-photochemical quenching (NPQ), and photochemical quenching (qP) 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 (1×10^6 and 1×10^{10} CFU mL^{-1}) significantly decreased the stomatal aperture (Fig. 1).

Effects of yeast on leaf gas-exchange

The g_s and T_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 (1×10^6 and 1×10^{10} CFU mL^{-1}) ofyeast than in the controls (Fig. 2E). Underyeast 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_v/F_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_v/F_m and NPQ were observed between the yeast-treated and control plants (Fig. 3A, B). qP 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_s and P_n, T_r, and WUE on the 7th day

The relationship between P_n and g_s was well represented by a logarithmic function ($r^2 = 0.93$) (Fig. 4A). Fig. 4B shows a linear relationship between T_r and g_s ($r^2 = 0.99$) on the 7th day after spraying. Fig. 4C shows a relationship between WUE and g_s on the 7th day after spraying, wherein WUE increased linearly with decreasing g_s and reached a peak at approximately 10 mmol mol^{-1}, when g_s approached 20 mmol $m^{-2} s^{-1}$; however, when g_s 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_r and g_s 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 ($[Ca^{2+}]_{cyt}$) in guard cells (Klüsener 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

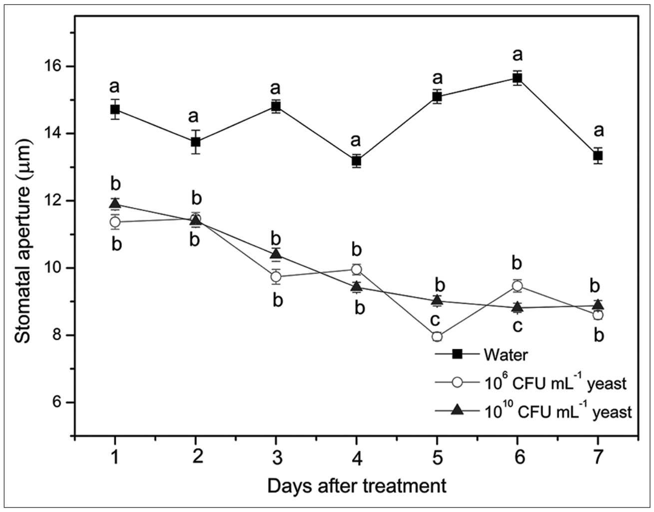

Fig 1. Changes in stomatal aperture of *Vicia faba* after treatment with two concentrations of yeast for 7 days. Error bars indicate ± SE (n=90). The different letters represent significant differences (P<0.05) and ns represents no significant difference (P>0.05).

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

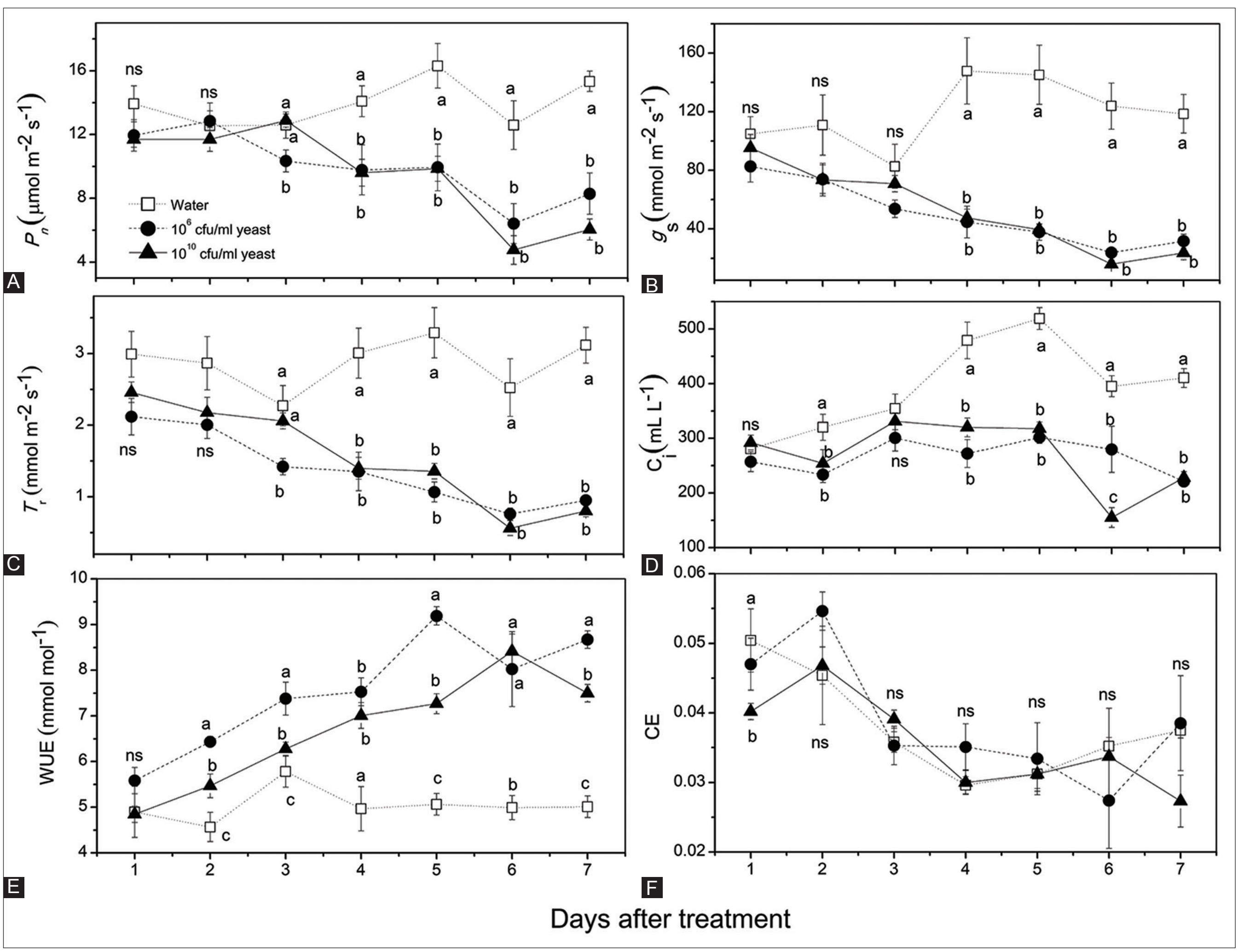

Fig 2. Changes in net photosynthetic rate, P_n(A), stomatal conductance, g_s (B), transpiration rate, T_r (C), intercellular CO_2 concentration, C_i (D), water use efficiency (WUE; P_n/T_r) (E) and Carboxylation efficiency (CE; P_n/C_i) (F) of *Vicia faba* after treatment with two concentrations of yeast 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).

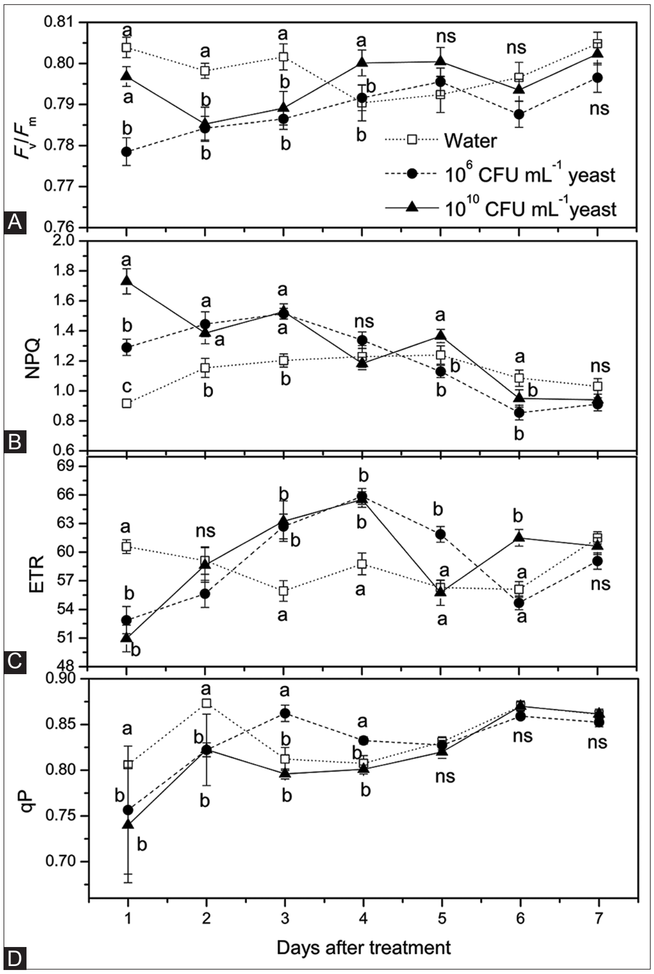

Fig 3. Changes in non-photochemical quenching, NPQ (A), maximum photochemical efficiency of PS II, F_v/F_m (B), photochemical quenching, qP (C) and electron transport rate, ETR (D) of *Vicia faba* after treatment with two concentrations of yeast for 7 days. Error bars indicate ± SE (n=8). The different letters represent significant differences (P<0.05) and ns represents no significant difference (P>0.05).

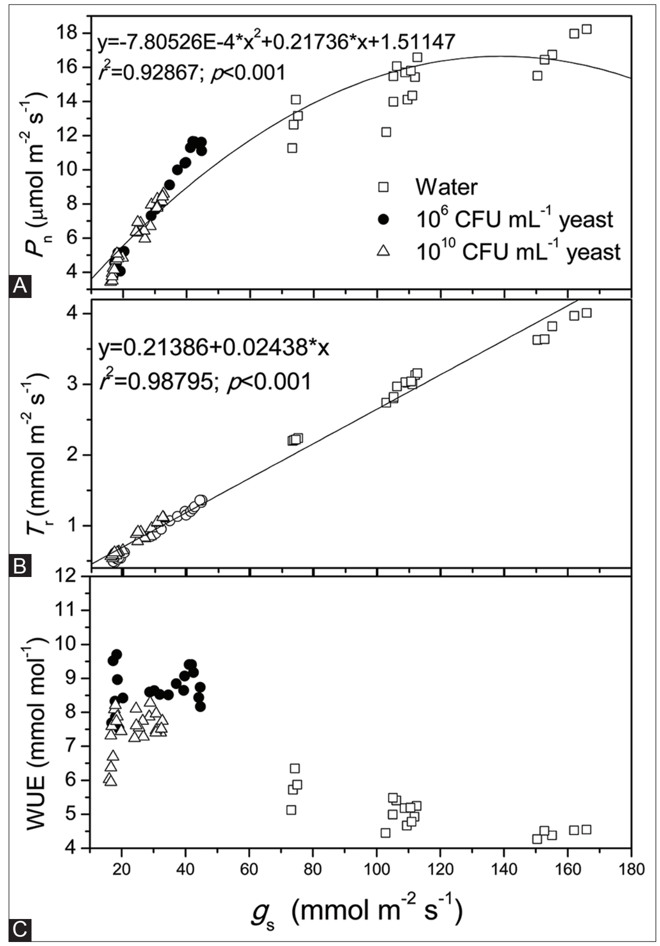

Fig 4. Correlations between net photosynthetic rate, P_n, transpiration rate, T_r, water use efficiency (WUE; P_n/T_r) and stomatal conductance, g_s in *Vicia faba* on the 7th day after spraying yeast.

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).

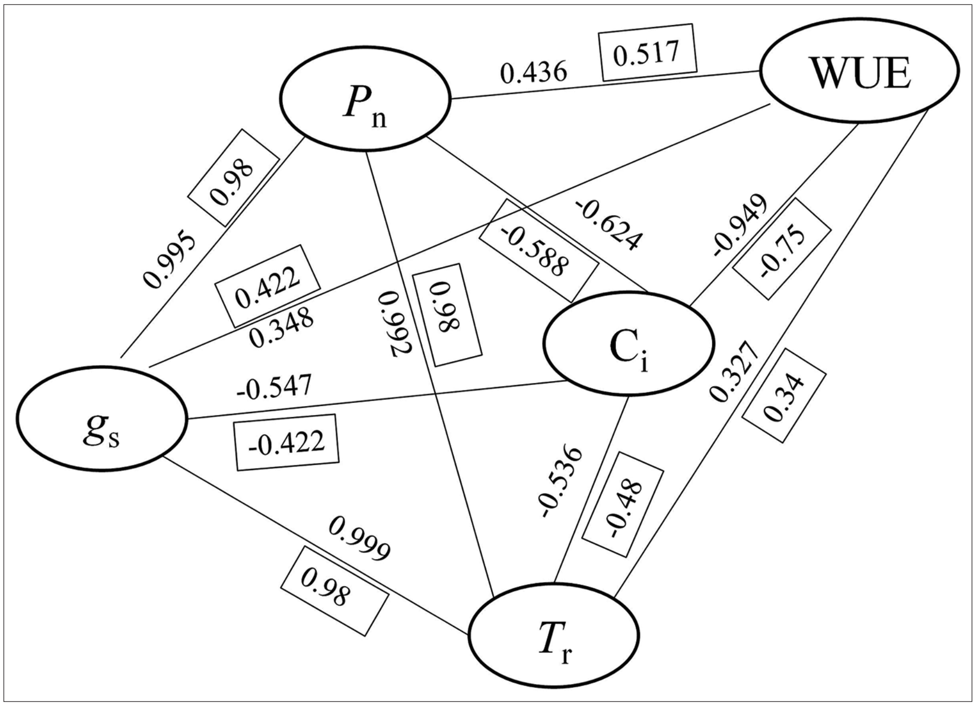

Fig 5. The relatioships between gas exchange parameters (net photosynthetic rate P_n, transpiration rate T_r, water use efficiency WUE, intercellular CO_2 concentration C_i and stomatal conductance g_s) of *Vicia faba* on the 7th day after spraying 1×10^6 and 1×10^{10} CFU mL^{-1}(in line rectangle) yeast.

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_2O 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 1×10^{10} CFU mL^{-1} induced more stomatal resistance than 1×10^6 CFU mL^{-1} of yeast, while the maximum WUE occurred at 1×10^6 CFU mL^{-1} of yeast. Therefore, as an antitranspirant, yeast was more effective at the concentration of 1×10^6 CFU mL^{-1} than at 1×10^{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 ChineseHerbal 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; GenxuanWang and SuiqiZhang 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. Phylloplane 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. Balestrini. 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_2O_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. Mori and Y. Murata. 2010. Yeast elicitor-induced stomatal closure and peroxidase-mediated ROS production in Arabidopsis. Plant Cell Physiol. 51 1915-1921.

Klüsener, B., J. J. Young, Y. Murata, G. J. Allen, I. C. Mori, V. Hugouvieux and J. I. Schroeder. 2002. Convergence of calcium signaling pathways of pathogenic elicitors and abscisic 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., W. 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.

Ortíz-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.