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Photosynthetic responses of wheat (*Triticum aestivum* L.) to combined effects of drought and exogenous methyl jasmonate

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Abstract

Drought stress limits wheat growth and productivity. The response of wheat (*Triticum aestivum* L.) to different water supply conditions (well-watered and drought-stressed) and exogenous methyl jasmonate (MeJA; 0 and 0.25 μM) was studied. The application of MeJA enhanced wheat adaptability to drought stress by physiological and metabolic adjustments. Drought stress reduced net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E), and water-use efficiency (WUE) in wheat. The application of exogenous MeJA decreased also g_s and E, but stimulated WUE. Meanwhile, MeJA mitigated the decline of P_N, g_s, and WUE induced by drought stress and midday depression by 6–183%. Both drought stress and exogenous MeJA induced stomatal closure, which improved water status and delayed plant senescence. MeJA enhanced the activities of superoxide dismutase, peroxidase, catalase, and reduced malondialdehyde content. P_N-PAR response curves showed that MeJA mitigated the decline of maximum P_N, apparent quantum yield, and saturation irradiance, and the increase of compensation irradiance. Drought stress and exogenous MeJA increased dark respiration rate and showed an additive effect. These results indicated that 0.25 μM MeJA enhanced the photosynthesis under drought stress mainly by improving the water status and antioxidant capacity of wheat.

Additional key words: antioxidant enzyme; gas exchange; stomatal behavior; water stress.

Introduction

Drought inhibits photosynthesis and decreases crop growth and productivity (Boyer 1982). Crop response to drought stress involves reduction in photosynthesis due to declined leaf expansion, impaired photosynthetic machinery, and premature leaf senescence (Wahid and Rasul 2005). Drought-induced stomatal closure limits the inflow of CO_2 into the leaves (Zheng *et al*. 2010) and reduces activities of Calvin cycle enzymes, such as Rubisco and phospho*enol*pyruvate carboxylase (Bota *et al*. 2004). Furthermore, drought stress leads to enhanced lipid peroxidation and the degradation of nucleic acids, proteins, and biomembranes (Berlett and Stadtman 1997). The reactive oxygen species (ROS), produced under drought stress, targets various organelles, including chloroplasts, mitochondria, and peroxisomes, which results in faster senescence or death of plants (Ma *et al*. 2013).

Wheat (*Triticum aestivum* L.) is one of the most important crops in China, planted mainly in semiarid and semihumid regions with limited rainfall. In 2009 and 2010, over 17 million ha of wheat suffered from severe drought, especially in the northwest region, where rainfall and irrigation were limited (Xia *et al*. 2012). Even in

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Abbreviations: AA – ascorbic acid; *ANOVA* – analysis of variance; CAT – catalase; DS – drought-stress treatment; DS+MJ – drought stress plus 0.25 μM MeJA treatment; E – transpiration rate; F_M – *ANOVA* of MeJA treatment; F_W – *ANOVA* of water treatment; $F_{W\times M}$ – *ANOVA* together with interactions between water and MeJA treatments; g_s – stomatal conductance; I_c – compensation irradiance; I_s – saturation irradiance; JA – jasmonic acid; L_T – leaf temperature; MDA – malondialdehyde; MeJA – methyl jasmonate; MJ – 0.25 μM MeJA treatment; P_{max} – maximum net photosynthetic rate; P_N – net photosynthetic rate; POD – peroxidase; R – roots; R_D – dark respiration rate; ROS – reactive oxygen species; S – shoots; S1 – flag leaf stage; S2 – flowering stage; S3 – filling stage; S4 – ripening stage; SOD – superoxide dismutase; WUE – water-use efficiency; WW – well watered plants; Ψ_w – leaf water potential; Φ – apparent quantum yield.
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humid regions, the growth of wheat usually suffers from seasonal or unexpected drought (Hu *et al*. 2012). Wheat is sensitive to drought at various growth stages and the yield is more readily affected by this stress (Ma *et al*. 2005).

Hormonal regulation plays an important role in drought tolerance of plants. Various effective substances, which are involved in drought tolerance at low concentrations, can be applied externally, but they are also produced internally (Morgan 1990). Under drought stress, the wheat growth improved after the exogenous application of appropriate concentrations of phytohormones, such as abscisic acid (Du *et al*. 2013), cytokinins (Gupta *et al*. 2000), and salicylic acid (Kang *et al*. 2012). Besides, it is well proved that application of exogenous jasmonates can improve drought tolerance of many species, such as soybean (Anjum *et al*. 2011), barley (Bandurska *et al*. 2003), cauliflower (Wu *et al*. 2012), and strawberry (Wang 1999). MeJA regulates many aspects of plant development, including seed germination, root growth, flowering, fruit ripening, and senescence (Wasternack 2007). MeJA is involved also in plants response to biotic and abiotic stresses (Avanci *et al*. 2010). The concentrations of endogenous jasmonic acid (JA) increased when plants were exposed to drought stress (Mahouachi *et al*. 2007). In addition, exogenous applications of MeJA can strengthen the dehydration tolerance of plants under drought stress caused by polyethylene glycol (Li and Staden 1998) and withholding water (Wang *et al*. 1999).

MeJA alleviated the influence of drought by promoting stomatal closure, which was induced by generation of H_2O_2 (Suhita *et al*. 2004). Exogenous applications of jasmonates could improve the resistance to many kinds of abiotic stresses, such as salt stress (Tsonev *et al*. 1998), UV-B stress (Liu *et al*. 2012), chilling stress (Cao *et al*. 2009), and heat stress (Ding *et al*. 2001), by activating the expression of signaling genes. However, few studies have focused on the mechanism of plant response to stresses mediated by exogenous MeJA. The present study was carried out to examine if exogenous MeJA alleviates the harmful effects of drought in wheat. For this purpose, we investigated changes of gas exchange, dry matter production, and associated metabolic changes to understand drought-tolerance mechanism.

Materials and methods

Growth conditions: The experiments were conducted at Henan Agricultural University, China (34°47′N, 113°38′E) from October 2011 to June 2012. The climate was characterized by a mean monthly air temperature of 8.1ºC and rainfall of 40 mm, which occured from October to May. Uniform seeds (15) of *Triticum aestivum* L., var. Zhoumai 18, were sown into prepared soil in round plastic pots (25 cm in diameter and 28 cm deep), which were buried 28 cm deep in field soil with a piece of plastic cloth under the pot bottom. Prepared soil was air-dried and filled into the pots after removing gravel, debris, and weeds, containing available nitrogen of 49.1 mg kg^{-1}, phosphorus of 47.4 mg kg^{-1}, potassium of 101.3 mg kg^{-1}, and 1.71% of soil organic matter. Each plastic pot was filled with 10 kg of the prepared soil and received 1.5 g of the compound fertilizer ($N:P_2O_5:K_2O$, 20:25:5). After the emergence, seedlings were thinned to a number of 10 per pot and allowed to grow under open-field conditions.

Drought and MeJA treatment: Drought treatment was initiated by withholding water on 1 April, 2012, when wheat was more vulnerable to water deficiency. Two water-supply regimes, 85–80% and 55–50% of field water capacity, were designated as well-watered (WW) and drought-stressed (DS) group, respectively. The average soil volumetric water contents were kept at 23.6 ± 0.8% in WW group and 14.2 ± 0.9% in DS group, respectively. Until plant maturity, the pots were weighed every other day and rewatered after 18:00 h if necessary to replenish the amount of water transpired. To avoid the disturbance from rainfall, plants were covered with a PVC sheet during the rain. At flag leaf stage (S1), MeJA (*Sigma*) was applied on the top of canopy at the concentrations of 0 or 0.25 μM in 0.5% Tween-20 solution. There were four treatments in our study with 15 replications per treatment:

WW	Control
MJ	0.25 μM MeJA
DS	Drought stress
DS+MJ	Drought stress + 0.25 μM MeJA

Each index was assayed by using 3 replications per treatment in one stage.

S1	Flag leaf stage
S2	Flowering stage
S3	Filling stage
S4	Ripening stage

Gas-exchange and P_N-PAR response curves: Fully expanded flag leaves were sampled using two portable photosynthesis systems (*LI-6400, LI-COR*, Lincoln, NE, USA). P_N-PAR response curves were measured at 2,000; 1,500; 1,000; 800, 500, 200, 100, 50, 20, and 0 μmol(photon) m^{-2} s^{-1} of PAR under uniform conditions [ambient CO_2 concentration of 330–360 μmol(CO_2) mol^{-1}, leaf temperature (L_T) of 28ºC, and 50–55% relative humidity inside the leaf chamber] at 9:00–11:30 h (local time) on two sunny days in every stage. Both photosynthetic gas exchange and stomatal conductance (g_s) were determined at 1,000 μmol(photon) m^{-2} s^{-1} of PAR under uniform conditions as P_N-PAR response curves

measurement. The instantaneous WUE was calculated as the ratio P_N/E. Diurnal changes of photosynthesis were determined with natural illumination under uniform conditions as P_N-PAR response curves measurements at 8:00–18:00 h (local time).

P_N-PAR response curves were fitted to a model using the modified equilateral hyperbola regression of *SPSS* software:

$$P_N = \Phi \frac{1 - AI}{1 + BI} I - R_D \quad (1)$$

where Φ is the initial slope of P_N-PAR response curve, defined as the apparent quantum yield. A and B were coefficients; I was PAR; and R_D was dark respiration rate. If $A = 0$ and $B = \Phi/P_{max}$, model 1 is the same as equilateral hyperbola model (model 2; Baly 1935):

$$P_N = \frac{\Phi I P_{max}}{\Phi I + P_{max}} - R_D \quad (2)$$

where P_{max} is the maximum net photosynthetic rate. The first derivative of model 2 is:

$$P'_N = \Phi \frac{1 - 2AI - ABI^2}{(1 + BI)^2} \quad (3)$$

When model 3 is set equal to 0, the I_s can be calculated as:

$$I_s = \frac{\sqrt{((A + B)/A} - 1}{B} \quad (4)$$

$$P_{max} = \Phi \left(\frac{\sqrt{A + B} - \sqrt{A}}{B} \right)^2 - R_D \quad (5)$$

R_D was measured in the dark (PAR = 0 μmol m^{-2} s^{-1} for at least 5 min). Linear regressions of irradiance and P_N over the range of 0–200 μmol(photon) m^{-2} s^{-1} of PAR were applied to determine I_c, and Φ (Yin *et al.* 2006). I_s and P_{max} were estimated from models 4 and 5.

Stomatal opening: 5 pieces from the flag leaf were taken and the leaf lower epidermis was fixed onto transparent tape. The leaf fragment was then shaved off mesophyll cell by using surgical blades and laid aside it on a glass slide. Two drops of absolute ethanol (containing 1% basic fuchsin) were applied before covering coverslip in order to fix and stain stomatal cells. Photographs were taken by a camera equipped light microscope (*Nikon 104*, *Nikon,* Japan). For each piece of sample, ten stomatal apertures were chosen to measure the stomatal length, width, and area. Then, the average value was taken as a value of the sample. The measurement was replicated three times.

Leaf water potential (Ψ_w) was determined on the flag leaves by using the water potential system (*Psypro*, *WESCOR*, USA) at 11:30 h. Five flag leaves were chosen and the measurement was replicated three times.

Biochemical analysis: For the biochemical analysis, a spectrophotometer (*TU-1810*, *PGENERAL*, China) was used. A leaf powder was extracted with 0.1 M PBS (pH 6.8), 0.1 M PBS (pH 7.8), 0.05 M PBS (pH 5.5), 0.05 M Tris-HCL (pH 7.0) and 10% TCA buffer after being ground in liquid nitrogen with a small mortar and pestle in analysis of protein, SOD, POD, CAT and MDA, respectively. Protein content was determined using Coomassie Brilliant Blue G-250 method (Bradford 1976). Superoxide dismutase (SOD, EC 1.15.1.1) was assayed by measuring its ability to inhibit photochemical reduction of nitroblue tetrazolium (NBT) chloride. One unit of SOD activity was defined as the amount of enzyme required to cause a 50% inhibition of the reduction at 560 nm (Beauchamp and Fridovich 1971). Peroxidase (POD, EC 1.11.1.7) activity was determined specifically with guaiacol at 470 nm. The activity was expressed in units (guaiacol oxidation product per min) per mg of a leaf tissue (Egley *et al*. 1983). Catalase (CAT, EC 1.11.1.6) activity was assayed by measuring the rate of disappearance of H_2O_2 at 240 nm The activity was expressed in units (H_2O_2 decomposed per min) per mg of a leaf tissue (Cakmak and Marschner 1992). Malondialdehyde (MDA) was determined by using thiobarbituric acid method (Dhindsa *et al.* 1981).

Dry mass (DM): Plants were harvested at their maturity stage, and the whole pot was separated into spikes, shoots (S), and roots (R). DM of all the parts were determined after 72 h in an oven at 85ºC. The ratio of R *vs*. S (R/S) was calcultated.

Statistical analyses were carried out by using the *SPSS* software (*version 13*, *SPSS*, Chicago, IL, USA). Differences between means of treatments were performed by the *Duncan*'s test with means considered significantly different at $p<0.05$.

Results

***P*$_N$-PAR response curves and gas exchange**: DS, exogenous MeJA, and the combination of these two factors affected significantly P_N-PAR response curves for the saturation irradiance (I_s) and the compensation irradiance (I_c). DS decreased I_s by 13.0–25.5%, but I_c increased by 84.8–169.5% from the S1 stage to S4 stage stage (Fig. 1). Moreover, DS sharply reduced P_{max} by 36.3–60.4%, while exogenous MeJA declined P_{max} by 13.2–14.8% before S3 stage stage, and elevated P_{max} by 6.8–7.4% after this stage (Table 1). Φ of the flag leaves was

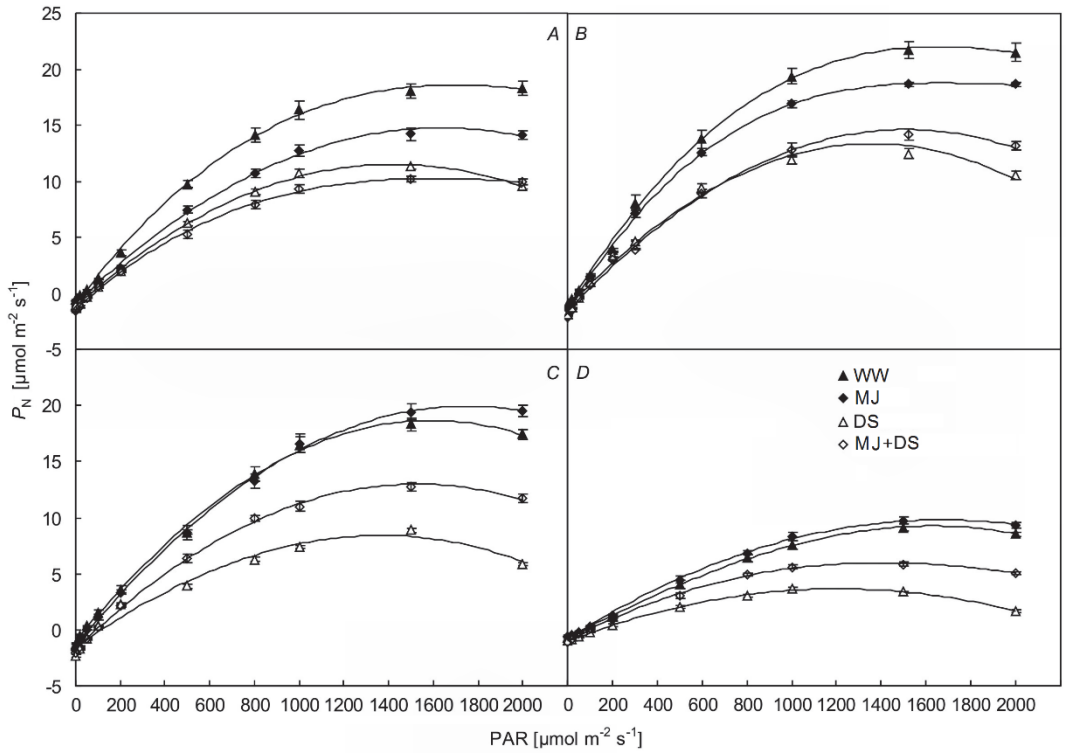

Fig. 1. Effects of exogenous MeJA (MJ, ■), drought stress (DS, △), and its interaction (□) on P_N-PAR response curves for four stages: *A*, flag leaf stage; *B*, flowering stage; *C*, filling stage; *D*, ripening stage). WW (▲) represents control. Means ± SE of three replicates.

Table 1. Saturation irradiance (I_s), compensation irradiance (I_c), the maximum net photosyntheticrate (P_{max}), apparent quantum yield (Φ), and dark respiration rate (R_D) of four stages (S1–S4) at different water supply regimes (well-watered and drought-stressed) and different concentrations of MeJA (0 and 0.25 μM). All the values are means of three replications. Means ± SE, n = 3, *p<0.05, **p<0.01. F_W – *ANOVA* of water treatment; F_M – *ANOVA* of MeJA treatment; $F_{W\times M}$ – *ANOVA* together with interactions between water and MeJA treatments. S1 – flag leaf stage; S2 – flowering stage; S3 – filling stage; S4 – ripening stage; WW – control; MJ – 0.25 μM MeJA; DS – drought stress.

Treatment		I_s [μmol m^{-2} s^{-1}]	I_c [μmol m^{-2} s^{-1}]	P_{max} [μmol m^{-2} s^{-1}]	Φ [μmol μmol^{-1}]	R_D [μmol m^{-2} s^{-1}]
S1	WW	1680.7 ± 24.8^{a}	36.26 ± 1.42^{c}	18.68 ± 0.67^{a}	0.0267 ± 0.0011^{a}	0.95 ± 0.02^{b}
	MJ	1685.1 ± 37.4^{a}	53.94 ± 5.25^{b}	16.22 ± 1.80^{a}	0.0217 ± 0.0017^{b}	1.16 ± 0.18^{ab}
	DS	1438.1 ± 22.9^{b}	70.31 ± 5.26^{a}	11.90 ± 0.12^{b}	0.0179 ± 0.0009^{b}	1.22 ± 0.04^{ab}
	DS+MJ	1626.6 ± 35.7^{a}	80.52 ± 5.67^{a}	10.29 ± 0.27^{b}	0.0191 ± 0.0008^{b}	1.45 ± 0.04^{a}
S2	WW	1703.8 ± 7.4^{a}	38.81 ± 2.94^{c}	22.99 ± 0.73^{a}	0.0220 ± 0.0016^{a}	0.84 ± 0.05^{c}
	MJ	1689.8 ± 7.3^{a}	53.32 ± 3.38^{c}	19.59 ± 0.18^{b}	0.0213 ± 0.0008^{a}	1.12 ± 0.03^{b}
	DS	1482.6 ± 5.7^{c}	71.72 ± 5.57^{b}	12.80 ± 0.41^{d}	0.0171 ± 0.0004^{b}	1.20 ± 0.07^{ab}
	DS+MJ	1643.2 ± 15.0^{b}	91.37 ± 6.94^{a}	14.85 ± 0.54^{c}	0.0153 ± 0.0006^{b}	1.38 ± 0.06^{a}
S3	WW	1588.4 ± 19.2^{b}	43.25 ± 3.54^{c}	18.75 ± 0.57^{a}	0.0244 ± 0.0010^{a}	1.03 ± 0.06^{c}
	MJ	1742.2 ± 31.5^{a}	60.82 ± 5.83^{c}	20.02 ± 0.63^{a}	0.0240 ± 0.0013^{a}	1.43 ± 0.07^{b}
	DS	1373.6 ± 12.5^{c}	116.54 ± 10.09^{a}	8.48 ± 0.19^{c}	0.0139 ± 0.0003^{b}	1.55 ± 0.12^{b}
	DS+MJ	1545.3 ± 15.1^{b}	92.57 ± 6.02^{b}	12.66 ± 0.37^{b}	0.0218 ± 0.0006^{a}	1.92 ± 0.07^{a}
S4	WW	1634.5 ± 24.9^{a}	73.36 ± 3.83^{bc}	9.27 ± 0.30^{a}	0.0108 ± 0.0008^{a}	0.78 ± 0.02^{b}
	MJ	1647.5 ± 31.2^{a}	63.81 ± 3.84^{c}	9.96 ± 0.30^{a}	0.0118 ± 0.0005^{a}	0.74 ± 0.02^{b}
	DS	1216.9 ± 5.6^{c}	141.71 ± 11.59^{a}	3.67 ± 0.11^{c}	0.0076 ± 0.0002^{a}	1.01 ± 0.05^{a}
	DS+MJ	1411.9 ± 12.6^{b}	97.64 ± 9.65^{b}	6.00 ± 0.18^{b}	0.0108 ± 0.0005^{b}	1.00 ± 0.06^{a}
F_W		218.18**	647.34**	766.32**	749.64**	149.37**
F_M		4.27*	184.83**	2.36	0.03	53.06**
$F_{W\times M}$		34.05**	75.70**	30.056**	40.11**	0.1

Table 2. Stomatal conductance (g_s), leaf temperature (L_T), net photosynthetic rate (P_N), transpiration rate (E), and water-use efficiency (WUE) of four stages (S1–S4) at different water supply regimes (well-watered and drought-stressed) and different concentrations of MeJA (0 and 0.25 μM). All the values are means of three replications. Means ± SE, $n = 3$, $^{*}p<0.05$, $^{**}p<0.01$. F_W – *ANOVA* of water treatment; F_M – *ANOVA* of MeJA treatment; $F_{W\times M}$ – *ANOVA* together with interactions between water and MeJA treatments. S1 – flag leaf stage; S2 – flowering stage; S3 – filling stage; S4 – ripening stage; WW – control; MJ – 0.25 μM MeJA; DS – drought stress.

Treatment		g_s [mol m^{-2} s^{-1}]	L_T [°C]	P_N [μmol m^{-2} s^{-1}]	E [mmol m^{-2} s^{-1}]	WUE [μmol μmol^{-1}]
S1	WW	0.546 ± 0.014^{a}	26.33 ± 0.210^{c}	16.33 ± 0.815^{a}	5.283 ± 0.343^{a}	3.098 ± 0.059^{b}
	MJ	0.285 ± 0.024^{b}	29.51 ± 0.278^{a}	12.71 ± 0.555^{b}	3.319 ± 0.165^{b}	3.833 ± 0.035^{a}
	DS	0.378 ± 0.019^{c}	27.96 ± 0.280^{b}	10.66 ± 0.375^{c}	3.747 ± 0.126^{bc}	2.858 ± 0.189^{b}
	DS+MJ	0.203 ± 0.014^{d}	30.14 ± 0.345^{a}	9.31 ± 0.340^{c}	2.587 ± 0.227^{c}	3.633 ± 0.210^{a}
S2	WW	0.661 ± 0.022^{a}	27.77 ± 0.186^{c}	19.39 ± 0.669^{a}	5.588 ± 0.151^{a}	3.471 ± 0.087^{bc}
	MJ	0.428 ± 0.021^{b}	29.42 ± 0.350^{b}	16.94 ± 0.314^{b}	4.369 ± 0.139^{b}	3.880 ± 0.067^{ab}
	DS	0.485 ± 0.015^{b}	30.93 ± 0.188^{a}	11.97 ± 0.378^{c}	3.927 ± 0.118^{b}	3.048 ± 0.045^{c}
	DS+MJ	0.463 ± 0.016^{b}	31.72 ± 0.245^{a}	12.83 ± 0.585^{c}	3.184 ± 0.209^{c}	4.060 ± 0.275^{a}
S3	WW	0.377 ± 0.011^{a}	29.75 ± 0.633^{c}	16.50 ± 0.651^{a}	4.892 ± 0.163^{a}	3.373 ± 0.073^{b}
	MJ	0.310 ± 0.014^{b}	31.31 ± 0.387^{bc}	16.61 ± 0.816^{a}	4.194 ± 0.132^{b}	3.960 ± 0.072^{a}
	DS	0.216 ± 0.010^{d}	34.36 ± 0.984^{a}	7.38 ± 0.160^{c}	2.636 ± 0.149^{c}	2.814 ± 0.098^{c}
	DS+MJ	0.267 ± 0.011^{c}	33.37 ± 0.444^{ab}	11.02 ± 0.406^{b}	2.599 ± 0.140^{c}	4.248 ± 0.194^{a}
S4	WW	0.205 ± 0.010^{a}	31.28 ± 0.211^{c}	7.55 ± 0.334^{a}	2.649 ± 0.086^{a}	2.849 ± 0.038^{c}
	MJ	0.223 ± 0.009^{a}	32.09 ± 0.206^{c}	8.32 ± 0.400^{a}	2.433 ± 0.054^{a}	3.420 ± 0.152^{b}
	DS	0.040 ± 0.006^{c}	35.65 ± 0.415^{a}	3.63 ± 0.114^{c}	1.435 ± 0.071^{b}	2.535 ± 0.046^{c}
	DS+MJ	0.129 ± 0.013^{b}	34.01 ± 0.347^{b}	5.60 ± 0.162^{b}	1.446 ± 0.079^{b}	3.889 ± 0.194^{a}
F_W		202.719**	202.719**	457.433**	297.827**	2.14
F_M		100.065**	100.065**	0.002	86.573**	159.63**
$F_{W\times M}$		65.653**	65.653**	27.497**	11.265**	17.500**

reduced by 30.0–61.8% in DS plants during the whole S3 stage. However, exogenous MeJA mitigated significantly Φ reduction under DS by increasing the Φ by 18.0–45.2%. DS and exogenous MeJA enhanced significantly the R_D by 28.4–50.5% and 5.1–38.3%, respectively. In addition, DS and exogenous MeJA diminished g_s significantly by 26.2–80.5% and 17.8–47.8%, respectively, and showed an apparent synergistic effect (Table 2). Application of MeJA and DS increased L_T by 2.6–12.1% and 6.2–15.5%, respectively. They showed an additive effect due to the lower E. DS reduced significantly the P_N by 34.7–55.3%. Exogenous applications of MeJA decreased the P_N under WW, but increased the P_N under DS. In comparison with WW, DS decreased significantly WUE by 7.7–16.6%, while exogenous applications of MeJA increased WUE by 11.8–20.0%. In DS plants with exogenous MeJA, WUE reached the highest value (4.248 μmol μmol^{-1}).

Diurnal changes of P_N: A bimodal curve represented diurnal changes of P_N and an obvious midday-depression phenomenon occurred in flag leaves. However, decline of P_N in midday was accentuated by DS. Drought sharply decreased P_N at all the growth stages from S1 to S4. Exogenous MeJA lowered P_N significantly at S1 and S2 stages before 11:00 h, but the stimulation of P_N was found at S2 stage after 11:00 h and during the whole S3 to S4 stages. Exogenous MeJA alleviated the decline of P_N when the plants were exposed to DS and reduced the loss of photosynthetic CO_2 fixation caused by photosynthetic midday depression by 6–183%, thereby raising the productivity (Fig. 2).

Stomatal opening: At S1, the stomatal aperture was dependent on plant water status and on exogenous MeJA. At S4 stage, the stomatal aperture depended mainly on the water status. At S1, application of MeJA increased the stomatal length by 25.6% in WW plants, but the stomatal

Table 3. Changes of stomatal opening at flag leaf stage (S1) and ripening stage (S4) in different treatments. All the values are means of three replications. Means ± SE, $n = 3$. *Different letters* indicate significant differences between water treatments and MeJA treatment ($p<0.05$); 0.25 μM exogenous MeJA (MJ), drought stress (DS), and its interaction (DS+MJ). WW – control.

Stage	Treatment	Stomatal opening		
		Length [μm]	Width [μm]	Area [μm^2]
S1	WW	16.03 ± 1.33^{c}	1.53 ± 0.06^{a}	77.01 ± 0.25^{a}
	MJ	20.14 ± 0.79^{ab}	-	-
	DS	16.81 ± 0.90^{bc}	0.96 ± 0.02^{b}	50.67 ± 0.06^{b}
	DS+MJ	23.07 ± 1.34^{a}	-	-
S4	WW	14.02 ± 0.89^{b}	1.01 ± 0.10^{b}	44.46 ± 0.28^{b}
	MJ	14.18 ± 0.65^{b}	1.77 ± 0.11^{a}	78.81 ± 0.22^{a}
	DS	18.01 ± 0.46^{a}	-	-
	DS+MJ	16.16 ± 0.43^{ab}	0.89 ± 0.05^{b}	45.16 ± 0.07^{b}

width and area declined significantly. DS also increased the stomatal length by 4.9% compared with WW, but it declined the stomatal width and area by 37.3 and 34.2%, respectively. The additive effect of MeJA and DS increased the stomatal length by 43.9% and induced closure of stomata compared with WW (Table 3). At S4 stage, no differences were found between WW and MeJA treatment in the stomatal length under WW condition, but the application of MeJA increased the stomatal width and area by 75.2% and 77.3%, respectively. DS increased the stomatal length by 28.5%, but the stomatal width and area were declined significantly compared with WW; while application of MeJA alleviated the increase of stomatal length induced by drought, especially the decline of stomatal width and area.

DM and R/S ratio: Water-supply regimes, MeJA concentrations, and their interaction affected significantly DM of spikes, shoots, and roots. Total DM decreased significantly as a result of DS, while exogenous MeJA alleviated the declines of DM when wheat seedlings were exposed to DS. DM allocation to shoots decreased, whereas the allocation in roots increased along with DS. As such, the R/S ratio increased by 27%. However, exogenous MeJA reduced the increase to 9% in comparison with WW (Fig. 3).

Discussion

There are two critical periods of water requirement in the life cycle of wheat – flag leaf stage and filling stage (Zhang *et al*. 1999). In the present study, DS was initiated from the S1 to S4 stages, including two critical periods of water requirement in wheat. Our results showed that P_N decreased significantly when wheat seedlings were exposed to drought; this decline of P_N was a consequence of stomatal limitation (Yokota *et al*. 2002). Closure of stomata prevents transpiration to protect plants that suffered from acute water deficit (Cornic 2000), potentially affecting leaf turgor and water potential (Ludlow and Muchow 1990). Our results showed that exogenous MeJA induced the closure of stomata and this effects lasted to the mid-filling stage. The g_s showed similar trends. The relationship between MeJA and g_s was also observed by other groups (Wu *et al*. 2012). In addition, E decreased significantly by DS and exogenous MeJA, showing the apparent synergistic effect that resulted in elevating WUE during DS. Furthermore, both application of MeJA and DS elevated L_T and showed the additive effect, which resulted from stomatal closure. Because of improved water status in plants, exogenous MeJA mitigated the decline of P_N and g_s caused by DS. Furthermore, exogenous MeJA alleviated the decrease of Ψ_w induced by DS by means of stomata closure, suggesting that plant water status was mitigated because of declining E.

In wheat, stomatal and nonstomatal effects are involved in photoinhibition (Pons and Welschen 2003). It suppresses the growth, development, and productivity in wheat (Ma *et al*. 2006). DS increased the sensitivity to photoinhibition in wheat (Lu and Zhang 1998), which is consistent with the present study. The elevated L_T and Ψ_w induced the enhancement of vapor pressure differences between intercellular spaces of the leaf and the atmosphere. This causes a decrease in g_s in most plant species. High midday L_T can affect negatively chloroplast function. In direct sunlight, the strong light intensity, high temperatures, and relatively low humidity cause L_T rise up to 35 and 40°C (Ishida *et al*. 1999). These factors can

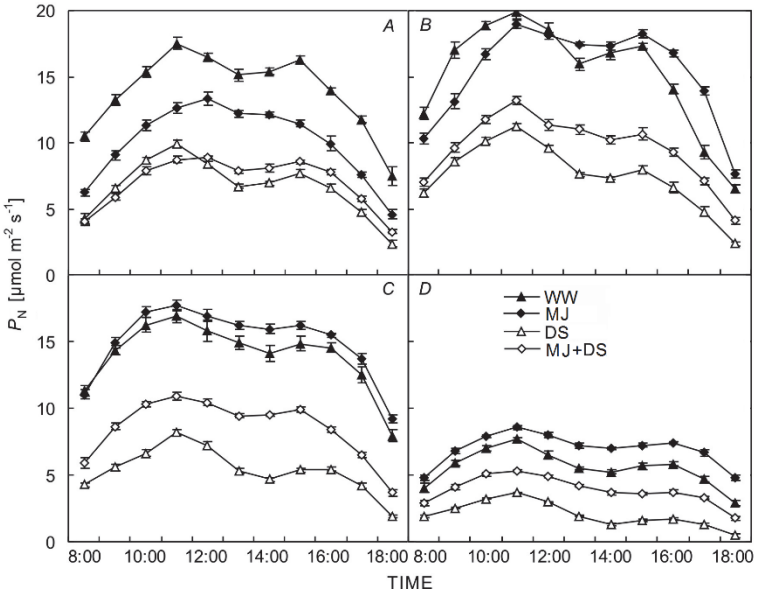

Fig. 2. Effects of exogenous MeJA (MJ, ■), drought stress (DS, △), and its interaction (□) on diurnal changes of photosynthetic rate (P_N) of four stages: *A*: flag leaf stage; *B*: flowering stage; *C*: filling stage; *D*: ripening stage. WW (▲) represents control. Means ± SE of three replicates.

be supraoptimal for photosynthesis in C_3 plants, unless those plants are adapted to hot climates (Berry and Bjorkman 1980). In the present study, the P_N diurnal changes decreased significantly by DS. Exogenous MeJA alleviated the declines of P_N when wheat seedlings were exposed to DS, which was dependent on the ameliorative water status due to the decline of E. These results were similar to the findings of Suhita *et al.* (2004).

DS caused the P_N decline at both saturating and subsaturating PAR. The P_{max}, I_s, and Φ decreased, while I_c and R_D increased when wheat seedlings were exposed to DS. The results were similar to studies of lovegrass plants (Colom and Vazzana 2003). However, exogenous MeJA decreased the P_{max} and Φ at the early-filling stage, and P_{max} increased at the middle and late stages of grain filling. This might occur because MeJA induced stomatal closure, which decreased the photosynthesis at early-filling stage, and water status improved photosynthesis at the middle and late stages. Decreasing I_s and increasing I_c would reduce the photosynthetic capacity, and the increase of R_D would make plants consume more assimilation products (Yin and Berninger 2006). Therefore, we concluded that assimilation decreased during the day and dissimilation increased during the night by DS, which suppressed plant growth and productivity. The results were in agreement with those of previous studies (Dizengremel and Gérant 1997). DS, as discussed above, was a main cause of photosynthesis reduction that often affects plant DM and the yield. In the present study, we found that drought stress affected the nutrient relations, such as less photosynthates were produced and more photosynthates were transported from shoot to root. Furthermore, exogenous MeJA prevented E by reducing the stomatal aperture, which mitigated the whole-plant water status.

Table 4. Changes of leaf water potential (Ψ_w), superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) activities, and malondialdehyde (MDA) content of four stages (S1–S4) at different water supply regimes (well-watered and drought-stressed) and different concentrations of MeJA (0 and 0.25 μM). All the values are means of three replications. Means ± SE, n = 3, $^{*}p<0.05$, $^{**}p<0.01$. F_W – *ANOVA* of water treatment; F_M – *ANOVA* of MeJA treatment; $F_{W\times M}$ – *ANOVA* together with interactions between water and MeJA treatments. S1 – flag leaf stage; S2 – flowering stage; S3 – filling stage; S4 – ripening stage; WW – control; MJ – 0.25 μM MeJA; DS – drought stress.

Treatment		Ψ_w [MPa]	SOD [unit mg^{-1}(protein)]	POD [unit mg^{-1}(protein)]	CAT [unit mg^{-1}(protein)]	MDA [μmol g^{-1}(FM)]
S1	WW	–1.776 ± 0.06^{a}	39.05 ± 1.748^{c}	23.56 ± 1.305^{c}	0.569 ± 0.025^{c}	13.74 ± 1.04^{b}
	MJ	–1.701 ± 0.04^{a}	48.81 ± 0.772^{ab}	34.63 ± 1.278^{a}	0.882 ± 0.020^{a}	14.17 ± 0.65^{b}
	DS	–2.873 ± 0.08^{c}	46.55 ± 0.732^{b}	28.91 ± 0.950^{b}	0.689 ± 0.016^{b}	21.92 ± 0.90^{a}
	DS+MJ	–2.579 ± 0.04^{b}	52.04 ± 0.609^{a}	35.24 ± 0.995^{a}	0.868 ± 0.021^{a}	21.55 ± 0.89^{a}
S2	WW	–1.822 ± 0.07^{b}	45.73 ± 0.495^{d}	24.55 ± 0.679^{d}	0.594 ± 0.032^{c}	17.63 ± 0.60^{c}
	MJ	–1.571 ± 0.04^{a}	54.85 ± 0.562^{b}	33.27 ± 0.622^{a}	0.932 ± 0.025^{a}	16.07 ± 0.28^{c}
	DS	–2.705 ± 0.05^{d}	47.63 ± 0.448^{c}	27.46 ± 0.501^{c}	0.760 ± 0.022^{b}	25.89 ± 0.63^{a}
	DS+MJ	–2.482 ± 0.03^{c}	57.84 ± 0.562^{a}	31.33 ± 0.370^{b}	0.868 ± 0.013^{a}	22.31 ± 0.83^{b}
S3	WW	–2.036 ± 0.08^{b}	33.27 ± 0.743^{c}	19.49 ± 0.643^{d}	0.523 ± 0.024^{d}	20.08 ± 0.70^{c}
	MJ	–1.755 ± 0.03^{a}	40.77 ± 1.005^{a}	29.97 ± 0.745^{a}	0.806 ± 0.020^{a}	17.52 ± 0.64^{c}
	DS	–2.880 ± 0.09^{d}	30.74 ± 0.365^{d}	23.13 ± 0.622^{c}	0.603 ± 0.013^{c}	35.83 ± 1.42^{a}
	DS+MJ	–2.627 ± 0.03^{c}	37.61 ± 0.630^{b}	26.25 ± 0.769^{b}	0.744 ± 0.015^{b}	28.52 ± 1.47^{b}
S4	WW	–2.317 ± 0.08^{b}	26.82 ± 0.539^{c}	8.97 ± 0.842^{c}	0.247 ± 0.033^{c}	28.64 ± 1.41^{c}
	MJ	–1.949 ± 0.08^{a}	32.46 ± 0.776^{a}	18.03 ± 1.026^{a}	0.616 ± 0.037^{a}	23.77 ± 0.95^{d}
	DS	–3.011 ± 0.08^{d}	23.70 ± 0.627^{d}	4.81 ± 0.905^{d}	0.117 ± 0.025^{d}	48.56 ± 1.92^{a}
	DS+MJ	–2.734 ± 0.05^{c}	29.37 ± 0.485^{b}	13.54 ± 0.675^{b}	0.475 ± 0.036^{b}	39.41 ± 1.52^{b}
F_W		807.97**	1.5	0.28	0.21	459.46**
F_M		68.12**	392.66**	329.10**	447.23**	45.19**
$F_{W\times M}$		0.09	1.55	26.08**	27.39**	7.56**

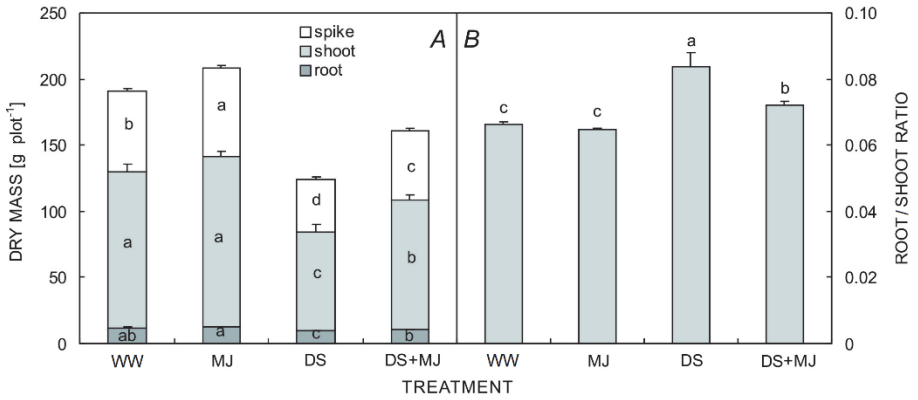

Fig. 3. Effects of 0.25 μM exogenous MeJA (MJ), drought stress (DS), and its interaction (DS+MJ) on dry mass (*A*), and root/shoot ratio (*B*) at the ripening stage. WW – control. Means ± SE of three replicates. *Different letters* indicate significant differences between water treatments and MeJA treatment (p<0.05).

DS also induced metabolic changes in the wheat seedlings. To protect cells against damage, antioxidant-defense enzymes are induced to clear the ROS and minimize the damage in plants (Wang 1999). Our results showed that DS stimulated the SOD, POD, and CAT activity at the early stage of grain filling, but these enzymes were inhibited at the late-filling period because of senescence induced by water deficit. There is no general consensus on the function of MeJA in antioxidative protection (Jubany-Marí *et al*. 2010). Our data showed that external applications of MeJA activated the antioxidant enzymes, including SOD, POD, and CAT, which resulted in decline in MDA content induced by DS.

In conclusion, the external application of MeJA induced stomatal closure to keep the plant at more favourable water status and increased the antioxidant ability under the drought condition. Thus, exogenous MeJA improved the photosynthesis and DM when wheat seedlings were exposed to drought.

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