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Evaluation of the concentration-response relationship between film antitranspirant and yield of rapeseed (*Brassica napus* L.) under drought

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ABSTRACT

Drought can cause large yield losses of rapeseed (*Brassica napus* L.), particularly during the flowering stage. Effective methods of crop management are required to improve the resistance to drought. Film antitranspirants (AT), as an effective method of crop management, can reduce water loss by forming a waterproof layer to block stomata mechanically. In our study, three pot experiments were conducted in the glasshouse at Harper Adams University in 2019–2020 to investigate the effect of two levels of irrigation - well-watered (WW) and water-stressed (WS); and AT at five concentrations - 0 (water), 0.25%, 0.5%, 0.75% and 1% at flowering stage on spring rapeseed. Results showed that water stress during the flowering stage depressed gas exchange significantly. Seed dry weight per plant reduced by an average of 70%, compared to WW control. Following AT application, stomatal conductance and photosynthesis rate were linearly associated with the concentration of AT for WW and WS plants in two of three experiments. With increasing AT concentrations, stomatal conductance was predicted to decrease ~1.4fold faster than photosynthesis rate. Some yield components showed an increase by AT application, however, the compensatory trade-off between pod number and seed number per pod accounted for the lack of a significant improvement in seed yield from AT-treated plants. Our results indicate that application of AT at flowering stage may be a potential method of mitigating the drought damage to rapeseed by blocking stomata, thereby sustaining seed yield. As AT-induced restrictions on leaf gas exchange are related to the concentrations, higher concentrations of film AT than those tested may be needed in future studies.

1. Introduction

With climate projections indicating more frequency and intensity of regional extreme events (such as droughts), negative impacts from drought on the yield of crops are more likely to take place in the future (IPCC et al., 2014). Drought is usually defined as the period when the water uptake by the plants exceeds the water attained by precipitation during the growing season from an agricultural point of view (Tuberosa, 2012). Great impacts of drought on the crops are leading to a large loss of crop production in arid and semi-arid regions around the world (Lesk et al., 2016).

Rapeseed (*Brassica napus* L.) (also known as canola or oilseed rape) has become the third most essential crop globally for edible oil, fodder, and biofuel production after soybean and palm oil (FAOSTAT, 2020). Previous studies have reported that rapeseed can be highly affected by water stress during the flowering stage in terms of seed yield and yield components such as pod number and seed number per pod (Johnston et al., 2002; Istanbulluoglu et al., 2010; Hess et al., 2015). Ahmadi and Bahrani (2009) compared the effect of water stress imposed at three different growth stages (flowering, pod development and seed filling stage) on rapeseed. It showed that plants under drought during the flowering stage experienced the largest reduction of seed yield and oil yield by 29.5% and 31.7%, respectively. Likewise, Tesfamariam et al. (2010) observed that seed yield was reduced by 30% and 51% over two growing seasons, respectively, when water stress occurred at flowering stage, compared to well-watered control treatments. Therefore, improving the resistance of rapeseed to drought especially at flowering stage is crucial in sustaining the seed yield when plants are subjected to water stress.

It is important to optimize water use efficiency by reducing water loss while sustaining the yield under drought conditions (AbdAllah et al., 2018). Film antitranspirants (AT) are emulsions sprayed on the surface of leaves that create a waterproof layer to block stomata and thereby decreasing the diffusion of water vapor (Gale and Poljakoff-Mayber, 1967; Kettlewell, 2014; Abdullah et al., 2015). The reduction of water loss from AT application is usually accompanied by

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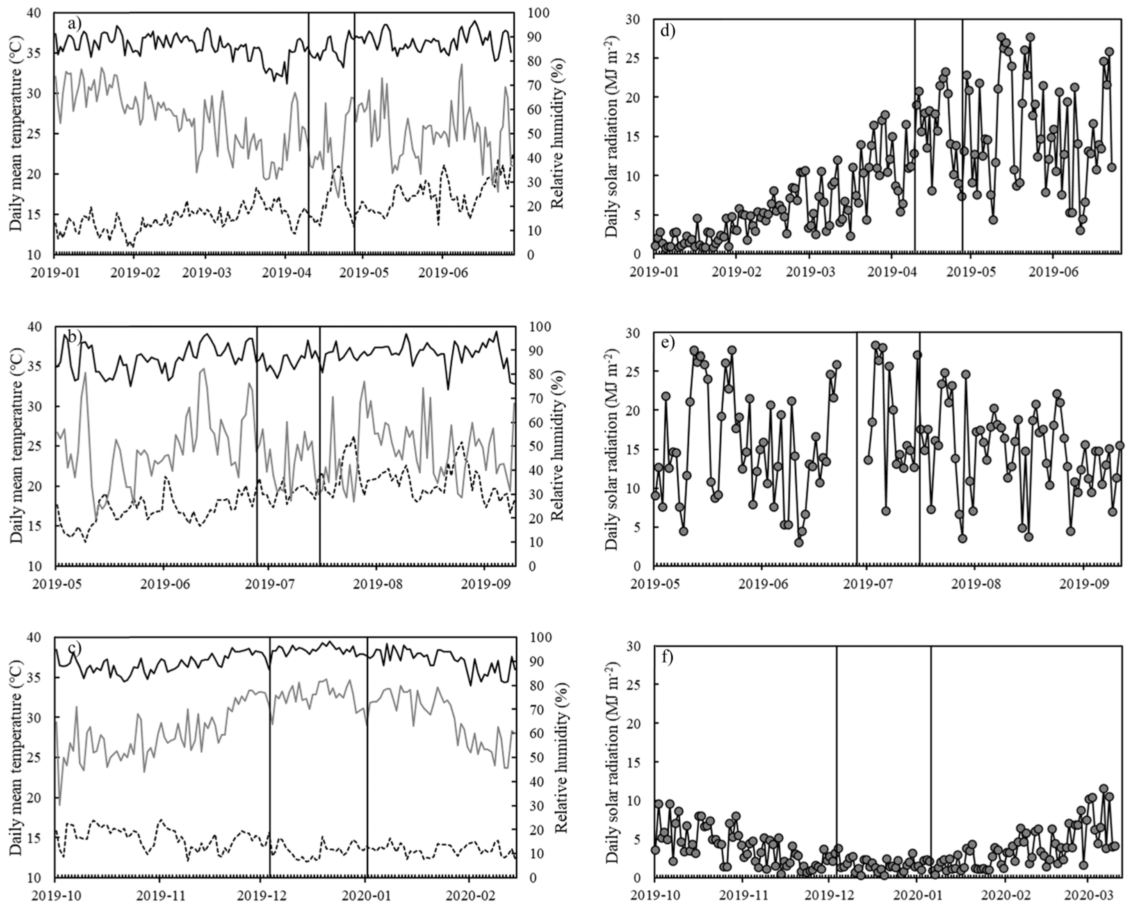

Fig. 1. Daily mean temperature (°C, ┄), maximum (━) and minmum (—) relative humidity (%) and daily solar radiation (MJ m^{-2}, -•-) during the growing season of rapeseed plants in Expt 1(a, d), Expt 2 (b, e) and Expt 3 (c, f), respectively. Two vertical lines represent the day of spraying film antitranspirant and the day when full irrigation was restarted in WS pots after drought, respectively.

the limitation on photosynthesis due to the low permeability of films to CO_2 entering the leaf, accordingly, film AT are mainly applied on ornamental species, in which photosynthesis is less important than the reduction of transpiration (Das and Raghavendra, 1979).

There is increasing evidence that the application of AT regulates plant water status and gas exchange to sustain the growth and yield of crops exposed to drought, such as tomato (AbdAllah et al., 2018), pea (Aldasoro et al., 2019) and sweet pepper (del Amor et al., 2010). There are only a few previous published papers investigating the mechanism of AT applied around the sensitive growth stage, that can mitigate the damage of drought on the yield when compared to unsprayed droughted plants (Kettlewell et al., 2010). For example, AT application relative to an unsprayed droughted control gave a rise in the grain yield of winter rapeseed by 39% (Faralli et al., 2016) and wheat by 11%– 16% (Weerasinghe et al., 2016; Mphande et al., 2021). Based on previous studies, however, the mechanism of how film AT mitigates the drought damage to crops during the critical growth stage still merits further investigation. In rapeseed, the correlations between gas exchange, leaf water status and yield components under drought conditions have not been widely explored. Furthermore, to our knowledge, effects of film AT applied at different concentrations on the yield and yield components of rapeseed under drought has not been reported in the literature.

Therefore, three pot experiments were carried out in our study with the aim of understanding the physiological mechanism of film AT applied on rapeseed plants at flowering stage and its effects on the final seed yield. The null hypothesis we tested is that there is no concentration-response of rapeseed to film AT on gas exchange, seed yield and yield components when plants are subjected to drought during the flowering stage.

2. Materials and methods

2.1. Planting material and research environment

Spring rapeseed seeds (*Brassica napus* L. var. Mirakel; NPZ-Lembke, Germany) were sown into 1-L pots containing ~0.5 kg John Innes No. 2 compost (loam, peat, coarse sand and base fertilizer, John Innes Manufacturers Association, Reading, UK) on 19th January 2019 in the glasshouse at Harper Adams University (HAU), at a rate of three seeds per pot with a spacing of 4 cm in Expt 1. Seedlings were thinned out at fourth-leaf stage and one plant was left in each pot. In Expts 2 and 3, seeds were sown in seedling trays at a rate of 25 seeds per tray on 7th May and 12th September 2019, respectively. One seedling was transplanted into each 7.5-L pot at fourth leaf stage, containing ~4.5 kg John Innes No. 2 compost and seedlings were watered immediately. The inter-pot spacing was ~25 cm and ~45 cm in Expt 1 and the other two experiments (Expts 2 and 3), respectively (Suppl. Fig. 1). Plants from three experiments were grown in the glasshouse at the light-dark photoperiod of 16–8 h, supplemented with high pressure sodium vapour lamps (Feilo Sylvania Europe Ltd, East Sussex, UK). Daily air temperature and relative humidity in the specific compartment for each experiment during the growing season were monitored by the AS3 Aspirated Screen Sensor and T200 logger installed in the glasshouse (TomTech (UK) Ltd, Spalding, UK). Daily solar radiation (MJ m^{-2}) was obtained from the weather station on campus. To estimate the effects of air temperature on rapeseed plants, thermal time in growing degree days (GDD) was estimated using Equation 1:

$$GDD = (T_m - T_b) \triangle t \quad 1)$$

Table 1
Treatments in Expt 1–3 (WW, well-watered; WS, water-stressed).

Expts	No.	Treatments	Concentration of AT (%)	Dose rate of AT (L ha^{-1})	Volume of AT (mL)	Volume of water (mL)
Expts 1&2	1	WW 0AT	0.00	0.00	0.00	100.00
	2	WS 0AT	0.00	0.00	0.00	100.00
	3	WW 0.25AT	0.25	0.60	0.25	99.75
	4	WS 0.25AT	0.25	0.60	0.25	99.75
	5	WW 0.5AT	0.50	1.30	0.50	99.50
	6	WS 0.5AT	0.50	1.30	0.50	99.50
	7	WW 0.75AT	0.75	1.90	0.75	99.25
	8	WS 0.75AT	0.75	1.90	0.75	99.25
	9	WW 1AT	1.00	2.50	1.00	99.00
	10	WS 1AT	1.00	2.50	1.00	99.00
Expt 3	1	WW 0AT	0.00	0.00	0.00	100.00
	2	WS 0AT	0.00	0.00	0.00	100.00
	3	WW 0.5AT	0.50	1.30	0.50	99.50
	4	WS 0.5AT	0.50	1.30	0.50	99.50
	5	WW 1AT	1.00	2.50	1.00	99.00
	6	WS 1AT	1.00	2.50	1.00	99.00

Table 2
Summary of irrigation management and film antitranspirant (AT) application in three experiments.

Events	Expt 1				Expt 2				Expt 3			
	GS	Date	DAP	GDD	GS	Date	DAP	GDD	GS	Date	DAP	GDD
Sowing		2019/01/19				2019/05/07				2019/09/12		
Irrigation stopped	GS61	2019/04/08	79	741	GS61	2019/06/24	48	549	GS63	2019/12/04	83	897
Target stress (30% AWC) achieved	GS61	2019/04/09	80	752	GS61	2019/06/26	50	576	GS63	2019/12/08	87	905
AT application	GS61	2019/04/10	81	762	GS61	2019/06/28	52	618	GS63	2019/12/04	83	897
Optimal irrigation re-started	GS69/71	2019/04/28	99	981	GS69/71	2019/07/16	70	889	GS69/71	2020/01/02	112	1147
Harvest	GS89	2019/06/26	158	1713	GS89	2019/09/09	126	1781	GS89	2020/03/09	179	1742

† AWC, available water content; GS, growth stage of plants (BBCH, Lancashire et al., 1991); DAP, days after planting; GDD, growing degree days (℃-d, base 5 ℃, Aiken et al., 2015).

Where T_m is the means of daily air temperature, T_b is the base temperature and $\triangle t$ is the number of days. In the present study, we used 5 °C as the base temperature (Aiken et al., 2015).

In Expt 1, plants were fertilised with ammonium nitrate (34.5% N, Yara Prilled N Fertiliser, Wynnstay, UK) at 0.15 g pot^{-1} and 0.3 g pot^{-1} before and after spraying AT, respectively. Nutrigrow triple-16 (Yara Universal 16 Fertiliser, Wynnstay, UK) was applied at 0.02 g pot^{-1} after spraying AT. In Expt 2, plants were fertilised with Nutrigrow triple-16 (0.7 g pot^{-1}) before spraying AT. Ammonium nitrate (1 g pot^{-1}) and Nutrigrow triple-16 (0.7 g pot^{-1}) were applied before spraying in Expt 3.

2.2. Experimental design

All the pots in three experiments were arranged in a randomised complete block design (RCBD) with two factors including two levels of irrigation (IR), well-watered (WW) and water stressed (WS); and film AT at different concentrations. There were five levels of AT in Expts 1 and 2 including 0 (water), 0.25%, 0.5%, 0.75% and 1%; and three levels in Expt 3 including 0 (water), 0.5% and 1%. There was a total of five blocks and 60 pots in Expts 1 and 2 while seven blocks and 42 pots in total in Expt 3 to increase the power of analysis of variance. Details of treatments in each experiment are shown in Table 1 and a schematic diagram of RCBD in Suppl. Fig. 1.

2.3. Irrigation supply

According to the soil water-retention curve (SWRC) of John Innes No. 2 compost (Saeed, 2008; unpublished data), the volumetric water content (VWC) at field capacity (FC) and permanent wilting point (PWP) of the compost were 45% and 7.5%, respectively. Available water content (AWC) of the compost was then calculated as the difference between FC and PWP, i.e., 37.5%. Across three experiments, WW pots were well-irrigated to ensure optimal growth throughout the growing season. In WS, drought was imposed over the flowering stage (BBCH 60–69, Lancashire et al., 1991) such that 70% of plant available water was allowed to deplete before irrigation was applied (i.e., 30% AWC). The inverted saucers of suitable size were placed beneath each pot in all three experiments to allow for free drainage from the bottom of the pots and to prevent any water uptake from the bench (Suppl. Fig. 1). The application of irrigation in three experiments is summarized in Table 2 and more details are as follows:

In Expts 1 and 2, VWC of WW and WS pots at a depth of ~8 cm was measured by ML2X theta probe (Delta T-devices, Cambridge, UK) every morning (9.00–10.00 am). In the afternoon (4.00–5.00 pm), WW pots were watered to ~FC using the hose pipe in Expt 1 while in Expt 2, WW pots were re-watered to 40% VWC (i.e., 87%). The amount of water required for WS pots in Expts 1 and 2, and WW pots in Expt 2 during the period of drought was calculated according to Equation 2 and 3, respectively.

$$WR_{ws} = (\theta_{AWC30} - \theta_m) \times \text{compost volume} \quad 2)$$

Where WR is water requirement in units of mL; θ_{AWC30} is VWC at the 30% AWC level between FC and PWP ($\theta = 18.8\%$); θ_m is measured VWC expressed as percentage; Compost volume for a 1-L pot is 675 and for a 7.5-L pot is 6100 in units of mL.

$$WR_{ww} = (\theta_{AWC87} - \theta_m) \times \text{compost volume} \quad 3)$$

Where θ_{AWC87} is VWC at the 87% AWC between FC and PWP ($\theta = 40\%$); Compost volume for a 7.5-L pot is 6100 in units of mL.

In Expt 3, the timing and irrigation management for both WW and WS pots were the same as Expt 2. In addition, extra water (30–50 mL) was added to WS pots to avoid large fluctuations in the dynamic change of soil moisture depending on the daily temperature through the weather forecast to estimate water loss through evapotranspiration. In the afternoon (4.30–5.00 pm), VWC was measured again to check if it

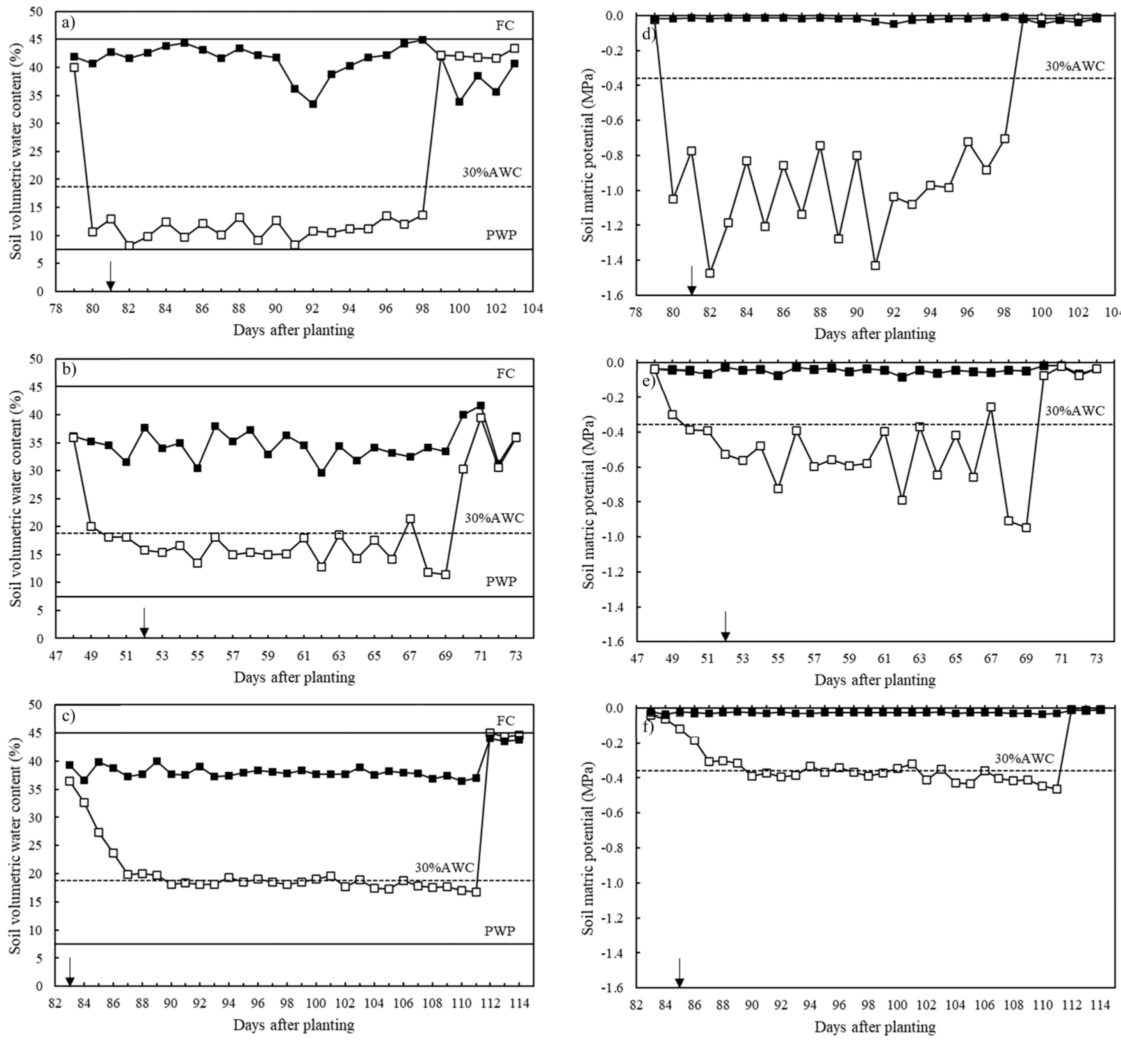

Fig. 2. Soil volumetric water content (VWC) (Expt 1- a; Expt 2- b; Expt 3- c) and soil matric potential (SMP) (Expt 1- d; Expt 2- e; Expt 3- f) of pots under well-watered (WW- -■-) and water stressed (WS- -□-) conditions before and after spraying film antitranspirant. Two horizontal closed lines represent field capacity (FC) and permanent wilting point (PWP) and dotted lines represent the target VWC/SMP of WS treatments, i.e., 30% available water content (30%AWC). Arrows represent the day of spraying film antitranspirant. Data are means of replicates (n = 5 in Expts 1 and 2; n = 7 in Expt 3).

was at the target VWC and re-watered if necessary.

2.4. *Antitranspirant application*

In Expts 1 and 2, film AT (Vapor Gard, a.i. di-1-*p* menthene 96%, VG. Miller Chemicals and Fertilizer, Hanover, USA) was applied when the target AWC 30% was achieved. In Expt 3, AT was applied on the same day when irrigation stopped in WS pots (Table 2). The adaxial surface of leaves was uniformly sprayed with water (0 AT) or a solution containing AT at an application rate of 250 L/ha using a custom-built pot sprayer (Flat Fan 110/002, 0.2 MPa) in an enclosed chamber located in a separate part of the glasshouse to simulate crop spraying in the field. Concentrations (%) and corresponding dose rates (L ha^{-1}) of AT in each experiment are shown in Table 1. We cleansed the tank and sprayed water only without plants in the chamber prior to sprays. Water was always sprayed first for the 0AT treatment, followed by AT solutions from the lowest to highest concentrations. To avoid cross-contamination between sprays of AT solutions, we washed the tank thoroughly with the brush until residues were removed. Given the high solubility of AT product, there was no need to use detergent.

2.5. *Gas exchange measurements*

Stomatal conductance (g_s, mol m^{-2} s^{-1}) and photosynthesis rate (A, μmol m^{-2} s^{-1}) were measured on the youngest fully expanded leaf of the top canopy (n = 3) using the LC*pro*-SD advanced portable photosynthesis system (ADC BioScientific Limited, UK) with a broad leaf chamber (6.25 cm^2) between 10.00 and 13.00 pm at three days after spraying (DAS) in three experiments. The temperature of leaf chamber was maintained at ~25 ℃, photosynthetically active photon flux density was 1044 μmol m^{-2} s^{-1} provided by an attached mixed Red/Blue LED array at a flow rate of 300 μmol s^{-1} through the leaf chamber. The CO_2 concentration of inlet air through the leaf chamber was ~380 ppm. All the data were recorded when steady-state photosynthesis was achieved after 3–5 mins. The flow check was calibrated before every use in three experiments to check that the cycle times were long enough for the gas in the analysis cell to become stable before the absorption was measured. Intrinsic water-use efficiency (WUEi, μmol (CO_2) mol $(H_2O)^{-1}$) was calculated from A divided by g_s.

2.6. *Yield and yield component measurements*

At maturity, all plants in Expts 2 and 3 were harvested while pod number per plant was counted. All the pods were then threshed by hand to determine seed yield based on dry matter. Seeds were dried in the oven at 60 ℃ to a constant weight for 72 h, recorded as seed dry weight per plant by using a 0.0001-g precision resolution balance. Seed number per plant was counted using "Analyse particles" programme in Image J software by taking photos of seeds spread out on white paper. Seed number per pod was then determined by dividing the seed number per plant by pod number and thousand-seed weight (TSW) was determined by the ratio of seed dry weight to seed number per plant and multiplied by 1000.

2.7. Statistical analysis

Data were analysed using GenStat 18th edition (VSN International, Hemel Hempstead, UK). Shapiro-Wilk and Levene tests were used for the estimation of normality and homogeneity of variance. Two-way analysis of variance (ANOVA) was employed to explore the AT concentration responses of plants to gas exchange and yield-related parameters with irrigation and AT as two factors with quadratic contrasts in Expt 1 (yield data excluded) and Expt 2, while with linear contrasts in Expt 3. Multiple comparisons were performed to compare the significant difference between treatments according to Tukey's test ($p = 0.05$) where there was significant interaction between irrigation and AT. Linear regression with groups analysis was used to test the relationships in g_s and A against AT concentrations, and between seed yield and yield components.

3. Results

3.1. Environmental conditions

The environmental conditions in the compartment of glasshouse varied among three experiments. The daily mean air temperature for the growing season was 16 ℃, 19 ℃ and 14 ℃ on average in Expts 1, 2 and 3, respectively (Fig. 1-a, b, c). It showed similar patterns in terms of maximum RH with an average of 87% for Expts 1% and 2%, and 91% for Expt 3, whereas the mean of minimum RH differed slightly, which was 52%, 48% and 65% in Expts 1, 2 and 3, respectively (Fig. 1-a, b, c). Plants received the same supplementation from artificial light. However, daily solar radiation (SR) differed substantially between experiments. The average of SR was 11 MJ m^{-2} in Expt 1 and 16 MJ m^{-2} in Expt 2 while only 4.2 MJ m^{-2} in Expt 3 (Fig. 1-d, e, f). The flowering started early in Expt 2 with the accumulation of 549 GDD (base 5 ℃) before onset, compared to Expts 1 and 3 (741 and 897 GDD, respectively). Concurrently, the duration of flowering stage varied, which was ~20 days in Expts 1 and 2, and more than a month in Expt 3 (Table 2). Despite these differences, the duration of whole growing season was similar between experiments with the range of 1713–1781 GDD.

3.2. Soil water status

Soil VWC and soil matric potential (SMP) as converted according to SWRC were shown in Fig. 2. In Expt 1, soil VWC (SMP) in the WW pots was maintained at the level of ~42% (SMP, −0.02 MPa) over the period of drought, whereas the average of soil VWC in WS was ~11% (−1 MPa) with substantial fluctuations around 8%– 14%, i.e., a SMP of between − 1.48 ~ − 0.70 MPa. It returned to the similar level as WW immediately after rewatering (Fig. 2-a, d). WS plants experienced less severe drought in Expts 2 and 3, when compared to Expt 1. Means of soil VWC (SMP) in WW were ~34% (−0.05 MPa) and ~38% (−0.03 MPa) in Expts 2 and 3, respectively. VWC in WS was ~16% (−0.58 MPa) and ~20% (−0.34 MPa) before rewatering, and after rewatering, soil VWC increased to ~35% (−0.05 MPa) and ~44% (−0.01 MPa) in Expts 2 (Fig. 2-b, d) and 3 (Fig. 2-c, f), respectively.

3.3. Gas exchange

Across all experiments, IR at flowering stage had significant effects on g_s, A and WUEi. The significant interaction IR×AT was observed in g_s in Expt 1, WUEi in Expt 2 and A in Expt 3 (Table 3). However, the difference between WW and WS was substantially larger than that between AT concentrations, and the interactions did not affect the very large mean effect of WS compared with WW. Thus, means of gas exchange between WW and WS groups were logically comparable. In Expts 1–3, g_s in WW was 0.33, 0.34 and 0.36 mol m^{-2} s^{-1}, respectively. WS plants exhibited a reduction in g_s by 89%, 63% and 62% as compared to WW plants (Fig. 3-a, b, c). The A in WW was 14.7, 15.1 and 16.3 μmol m^{-2} s^{-1} in Expts 1–3, respectively. When compared to WW plants, A in WS decreased by 72%, 23% and 21% in Expts 1–3, respectively (Fig. 3-d, e, f). WUEi was 45.9, 47.6 and 46.0 μmol (CO_2) mol (H_2O)$^{-1}$ under WW conditions in Expts 1–3. Water deficit increased WUEi of WS plants by 145%, 90% and 107%, when compared to WW control (Fig. 3-g, h, i) for these respective experiments.

Averaging WW and WS groups, the average g_s of unsprayed plants was ~0.3 mol m^{-2} s^{-1} in Expts 2 and 3. There was a linear relationship between g_s and AT concentrations, and g_s decreased by 12%– 19% and 9%– 24% relative to the control when AT was applied at the concentrations of 0.25%– 1% in Expt 2% and 0.5%– 1% in Expt 3, respectively. With a 1% increase in AT, g_s and A were predicted to decrease by 16% and 13% and by 25% and 17% in Expt 2 (Fig. 3-b, e) and Expt 3 (Fig. 3-c, f), respectively, as compared to corresponding mean of WW and WS control (0AT). For Expt 3 there was a significant linear interaction between IR and AT in g_s and A where g_s of WW declined greatly with increasing concentrations of AT, whereas WS only decreased slightly as AT concentrations increased (Fig. 3-c, f; Table 3). A similar trend was concurrently observed in A. In Expt 2, the interaction IRxAT was significant for WUEi, primarily accounted for the large deviation in 0.25AT and 1AT (Fig. 3-h; Table 3). Except that, there appeared to be no significant impacts from AT or IR×AT in other two experiments (Fig. 3-g, i).

Table 3
Probability values from *ANOVA* for stomatal conductance (g_s), photosynthesis rate (A) and intrinsic water use efficiency (WUEi) as affected by irrigation (IR), film antitranspirant (AT) in three experiments. Bold numbers indicate significant differences at $p < 0.05$.

Experiments	Factors	d.f.	*p values*		
			g_s	A	WUEi
Expt 1	Irrigation (IR)	1	**< 0.001**	**< 0.001**	**< 0.001**
	Antitranspirant (AT)	4	0.095	0.382	0.702
	Linear	1	0.687	0.141	0.993
	Quadratic	1	0.467	0.729	0.766
	Deviations	2	**0.028**	0.392	0.363
	IR×AT	4	**0.040**	0.352	0.927
	Linear	1	0.234	0.187	0.505
	Quadratic	1	0.192	0.414	0.997
	Deviations	2	**0.027**	0.363	0.815
Expt 2	Irrigation (IR)	1	**< 0.001**	**< 0.001**	**< 0.001**
	Antitranspirant (AT)	4	0.115	0.383	**0.008**
	Linear	1	**0.043**	**0.048**	0.578
	Quadratic	1	0.326	0.952	0.458
	Deviations	2	0.284	0.944	**0.002**
	IR×AT	4	0.071	0.888	**0.026**
	Linear	1	0.264	0.462	0.675
	Quadratic	1	0.863	0.869	0.789
	Deviations	2	**0.025**	0.763	**0.005**
Expt 3	Irrigations (IR)	2	**< 0.001**	**< 0.001**	**< 0.001**
	Antitranspirant (AT)	2	**0.042**	**0.009**	0.752
	Linear	1	**0.014**	**0.004**	0.524
	Deviations	1	0.633	0.210	0.697
	IR×AT	4	0.095	**0.019**	0.686
	Linear	1	**0.036**	**0.011**	0.442
	Deviations	1	0.656	0.176	0.702

3.4. Yield and yield components

At maturity, no reliable seed yield data were available from Expt 1

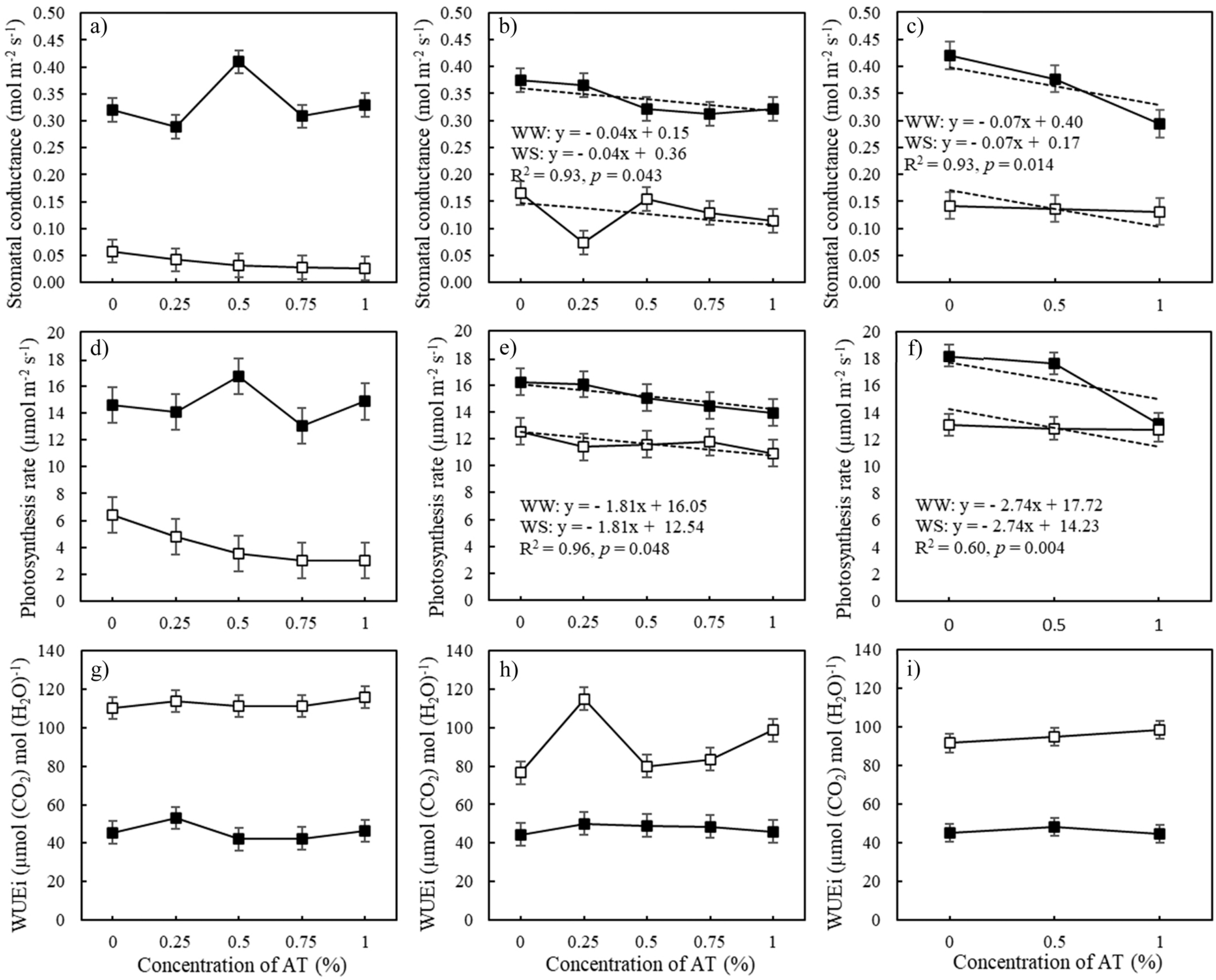

Fig. 3. Stomatal conductance (g_s), photosynthesis rate (A) and intrinsic water use efficiency (WUEi) of rapeseed plants at three days after spraying film antitranspirant (AT) at different concentrations under well-watered (WW, -■-) and water stressed (WS, -□-) conditions in Expt 1 (a, d, g), Expt 2 (b, e, h) and Expt 3 (c, f, i). The linear regression model with two groups (WW and WS) was fitted to g_s and A against concentrations of AT in Exps 2 (b, e) and 3 (c, f) where g_s and A had a significantly linear relationship with AT concentrations without significant interaction ($p < 0.05$, Table 3). Asterisks represent significant differences compared to corresponding unsprayed control according to Tukey's test ($p = 0.05$). Data are means of replicates (n = 5 in Expts 1 and 2; n = 7 in Expt 3) ± standard error of mean (SEM).

Table 4
Probability values from ANOVA for seed dry weight (SDW), pod number per plant (PP), seed number per pod (SP) and thousand-seed weight (TSW) as affected by irrigation and film antitranspirant in Expts 2 and 3. Bold numbers indicate significant differences at $p < 0.05$.

Expts	Factors	d.f.	*p* values			
			SDW	PP	SP	TSW
Expt 2	Irrigation (IR)	1	**< 0.001**	**< 0.001**	**0.017**	**< 0.001**
	Antitranspirant (AT)	4	0.601	**0.008**	0.065	0.061
	IR×AT	4	0.498	0.202	0.096	0.305
	Linear	1	0.195	**0.023**	0.459	**0.042**
	Quadratic	1	0.869	0.656	0.034	0.857
	Deviations	2	0.446	0.820	0.220	0.763
Expt 3	Irrigation (IR)	1	**< 0.001**	**< 0.001**	**0.002**	0.737
	Antitranspirant (AT)	2	0.642	0.486	0.053	0.519
	IR×AT	2	0.847	0.586	0.051	0.498
	Linear	1	0.583	0.570	0.918	0.288
	Deviations	1	0.875	0.391	**0.016**	0.618

due to the low quantity of pods. Across Expts 2 and 3, water deficit showed significantly negative impacts on seed yield and yield components, except for TSW in Expt 3 (Table 4). In Expt 2, plants grown under WW conditions had a production of ~19 g seeds and ~274 pods per plant with ~22 seeds per pod and ~3.3 g in TSW (Table 5). When compared to WW control, water deficit resulted in a reduction of 59% and 37% in seed dry weight and pod number, respectively, and to a lesser extent, 23% and 17% in seed number per pod and TSW, respectively (Fig. 4-a, c, e, g). Compared to Expt 2, seed yield and yield components were numerally lower in Expt 3. Plants under WW conditions produced ~16 g of seeds and ~405 pods per plant, as well as ~8 seeds per pod and ~5 g regarding TSW (Table 5). There was a consistent but greater reduction caused by water deficit in seed dry weight, pod number and seed number per pod by 81%, 77% and 38%, respectively, while the change in TSW was negligible (Fig. 4-b, d, f, h).

Averaging all concentrations of AT, consistent results from both experiments showed that there were no significant AT or IRxAT effects in seed dry weight (Table 4). However, responses in yield components were observed, albeit in varying degrees. In Expt 2, AT application

Table 5
The average of seed dry weight (SDW), pod number per plant (PP), seed number per pod (SP) and thousand-seed weight (TSW) as affected by the two main factors of irrigation including well-watered (WW) and water stressed (WS); and film antitranspirant (AT) including unsprayed AT (-AT) and sprayed AT (+AT) in Expts 2 and 3. The difference between WS and +AT in comparison to the corresponding control WW and -AT, respectively, were present as percentages, with positive values indicating increases and vice versa. Data are means with standard error of means (SEM), and asterisks represent the statistical significance according to Tukey's test ($p = 0.05$).

Expts	Factors	Treatments	SDW	Difference	PP	Difference	SP	Difference	TSW	Difference
Expt 2	Irrigation	WW	18.5		273.7		22.0		3.3	
		WS	7.6	-59%*	173.3	-37%*	17.1	-23%*	2.7	-17%*
		SEM	0.5		9.3		1.4		0.1	
	Antitranspirant	-AT	12.3		231.5		16.7		2.9	
		+AT	13.3	8%	221.5	-4%*	20.3	21%	3.1	5%
		SEM	0.8		14.8		2.2		0.1	
Expt 3	Irrigation	WW	15.8		405.0		8.2		4.7	
		WS	3.0	-81%*	92.7	-77%*	5.1	-38%*	4.8	2%
		SEM	1.8		34.6		0.7		0.2	
	Antitranspirant	-AT	7.7		233.0		5.1		4.9	
		+AT	10.3	35%	256.8	10%	7.4	45%	4.7	-4%
		SEM	2.2		42.4		0.8		0.2	

decreased pod number significantly while increased TSW with borderline significance by 4% and 5%, respectively (Table 5). Further, there was a significant linear interaction IRxAT in pod number per plant and TSW (Table 4). With an increase in AT concentrations from 0% to 1%, pod number of WW plants increased greatly but it decreased slightly for WS (Fig. 4-c). On the contrary, TSW in WW decreased marginally from 0% to 1% concentration of AT and the opposite was true in WS (Fig. 4-g). In Expt 3, borderline significances of AT and IRxAT interaction were observed in seed number per pod (Table 4).

Taking WW and WS as groups in regression, seed yield was highly associated with both pod number per plant and seed number per pod in Expts 2 and 3 (Fig. 5). There was a positive linear relationship of seed yield with pod number, explaining 64% of the variation with irrigation as a significant factor in Expt 2 (Fig. 5-a) while 87% in Expt 3 across WW and WS groups (Fig. 5-b). Similarly, seed yield was less positively but still significantly correlated to the number of seeds per pod in Expt 2 ($R^2 = 0.82$) and Expt 3 ($R^2 = 0.65$), taking differences between WW and WS plants into account (Fig. 5-c, d).

4. Discussion

4.1. *Water stress depressed stomatal conductance with concomitant decrease in photosynthesis rate*

Large variations between three experiments in gas exchange and thereby final seed yield production were accounted for by both the size of pots and various environmental conditions. Compared to 7.5-L pots used in Expts 2 and 3, 1-L pots exhibited large constraints on the root access to water, resulting in drastic fluctuations in soil water content (SMP) during the period of drought (Fig. 2). Despite daily air temperature and solar radiation varied slightly between Expts 1 and 2, plants in Expt 3 experienced a substantial reduction in both temperature and radiation during the growing season (Fig. 1). Given incident radiation and temperature having large effects on assimilation rate and the duration of flowering stage (Weymann et al., 2015), the difference of both factors in Expt 3 resulted in an elongation of the flowering stage, i.e., duration of drought imposed being longer than the former two experiments (Fig. 1; Table 2). In addition, water stress in Expt 3 appeared to be less severe, compared to Expts 1 and 2 (Fig. 2-c). Previous studies have shown that water stress can have detrimental effects on rapeseed plants and it is most pronounced during the flowering stage, followed by vegetative and seed filling stages (Tesfamariam et al., 2010). This is also supported by our studies involved with spring rapeseed. Gas exchange was consistently affected by water stress of varying degrees across three experiments. When compared to WW, water stress reduced g_s and A by 89% and 72%, respectively, in Expt 1 (~11% VWC); and to a lesser extent, by 62.5% and 22%, respectively, for Expts 2 and 3 on average (~18% VWC). The larger reduction of g_s and A which occurred in Expt 1 compared to the other two experiments was mainly attributed to the greater severity of water stress (Fig. 3). With the same type of compost (John Innes No. 2), Faralli et al. (2017) found that g_s and A of winter rapeseed reduced by 77.2% and 64.4% under severe water stress (~10% VWC), while under less severe water deficit (~20% VWC), there was a reduction of 48.5% and 41.0% in g_s and A, respectively. The difference in the reduction *of A* compared to our study, particularly under 20% VWC (41% vs 22%), might be explained by different species and/or the size of pots with restrictions on the root volume (Poorter et al., 2012). Across three experiments, WUEi from WS plants was 2–2.5 times higher than that from WW plants, regardless of AT treatments (Fig. 3-g, h, i). This indicates that assimilation rate, to certain degree, can be sustained with reduced water loss through stomata as a result of the higher proportional limitation of g_s compared to A as widely reported in the literature (Palliotti et al., 2013).

4.2. *The concentration-response of rapeseed to AT application under water stress in gas exchange*

Film AT inhibits the diffusion of gas by forming a film on the leaf to block the stomata physically, and thereby decreasing water loss from stomata as well as reducing photosynthetic efficiency (Gale and Hagan, 1966). In the present study, AT-treated plants showed a greater reduction in g_s than A (Fig. 3), thereby increasing WUEi, which is in agreement with findings of Faralli et al. (2016) on winter rapeseed and Abdullah et al. (2015) on wheat. Besides, g_s and A across WW and WS were linearly and negatively associated with the concentration of AT in two of three experiments in our study (Table 3). The g_s was projected to decrease 1.4 times faster than A with increasing concentrations of AT (Fig. 3-b, c, e, f). Those observations indicate that the suppression on gas exchange induced by AT was highly associated with the concentration of AT. Despite the significant effects from AT on WUEi was found only in Expt 2 (Fig. 3-h).

Gale and Poljakoff-Mayber (1967) reported that films formed by polyethylene, were distributed with unevenly varying thickness, and within some gaps as well as micropores. There is no doubt that the area covered by AT, i.e., leaf coverage, has direct impacts on the performance of AT. That is, the increase of leaf coverage induced by an increasing concentration of AT may result in further inhibition of gas exchange (Xiang et al., 2021). Faralli et al. (2017) compared three dose rates of AT, 1, 2 and 4 L/ha (i.e., 1%, 2% and 4%) on winter rapeseed in the field conditions. There was no additional reduction at higher dose rates (2 and 4 L/ha) compared to 1 L/ha. However, whether AT beyond 1% would inhibit gas exchange further still needs further investigation in specific crop varieties and under specific environmental conditions.

The g_s and A increased at some concentrations after AT application in

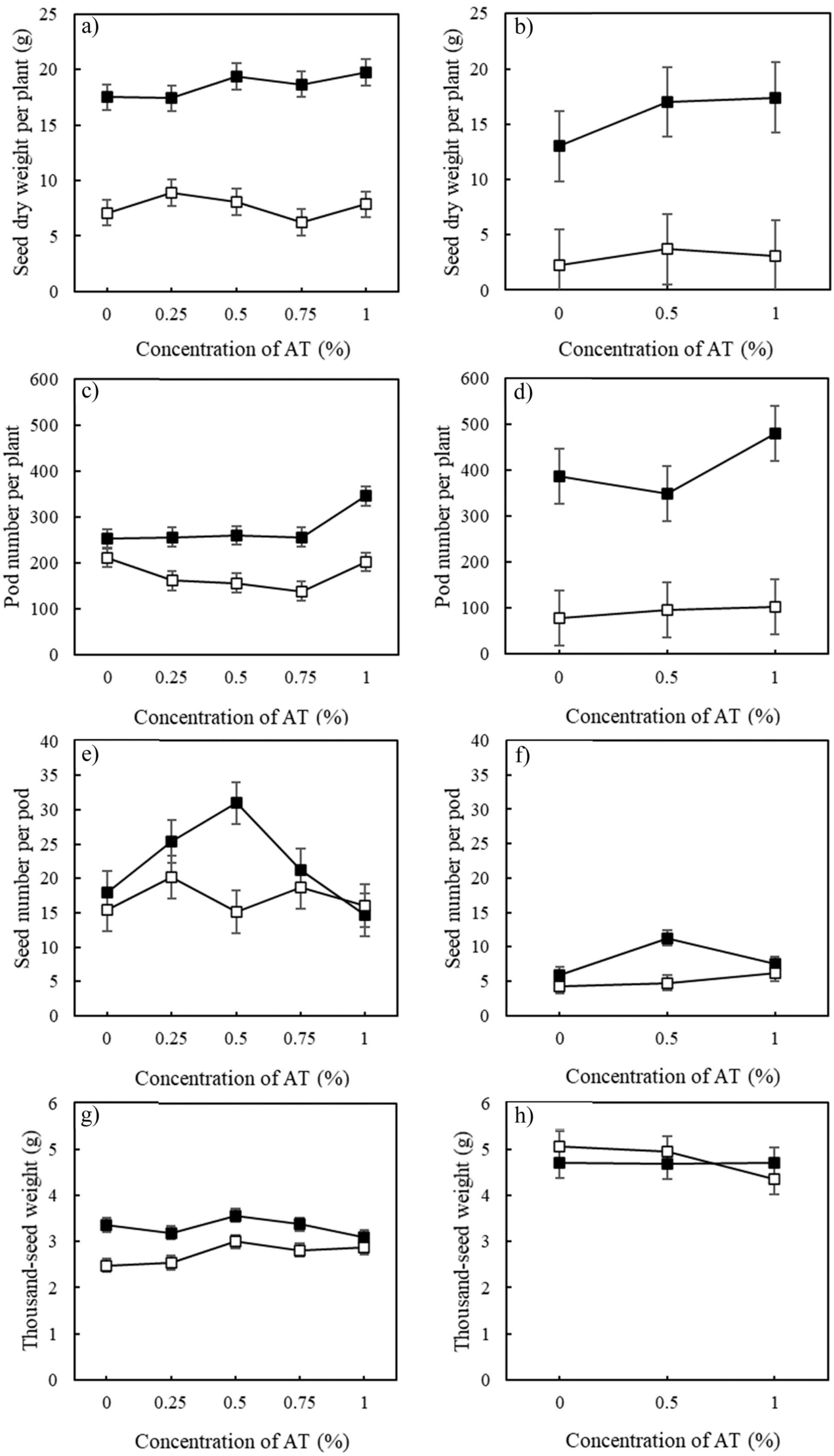

Fig. 4. Seed dry weight per plant and yield components of rapeseed plants under well-watered (WW, -■-) and water stressed (WS, -□-) conditions with application of film antitranspirants (AT) at different concentrations in Expt 2 (a, c, e, g) and Expt 3 (b, d, f, h). Data are means of replicates (n = 5 in Expt 2; n = 7 in Expt 3) ± standard error of mean (SEM).

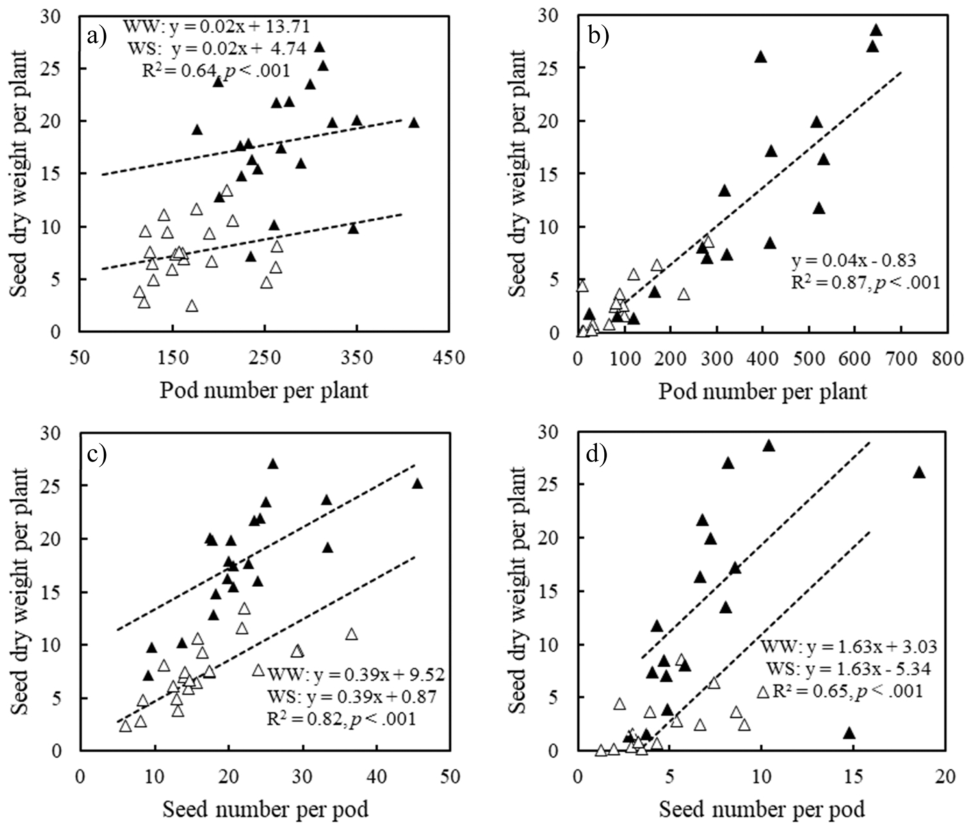

Fig. 5. Relationships between seed dry weight and yield components in Expt 2 (a, c) and Expt 3 (b, d). Parallel/common dotted lines were fitted with the linear regression model with/without irrigation as groups including well-watered (WW- ▲) and water-stressed (WS- Δ). Data are replicates in each treatment from Expt 2 (n = 5) and Expt 3 (n = 7).

WW groups of Expt 1 (Fig. 3-a, d), which is contrary to the general view about film AT that partial stomata blocked by AT physically would increase the resistance to diffusion of water vapor from stomata and consequently reduce *A* as discussed previously. The increased g_s could be caused in part by wider stomatal apertures induced by increasing leaf water potential as a result of AT application (Davenport et al., 1972). Further work is still needed to explore the stomatal size after application of AT with specific concentrations and the interaction between stomatal blockage by AT and the gas exchange processes involved.

4.3. Effects of water stress and AT application on seed yield and yield components

Compared to the corresponding WW control, a great yield loss occurred in WS plants (59% and 81% in Expts 2 and 3, respectively) when water deficit was imposed during the flowering stage (Table 5). The decrease in seed dry weight was associated with a reduction in seed number per plant (data not shown) related to both pod number and seed number per pod. This is also supported by strong correlations between seed dry weight and pod number per plant (Fig. 5-a, b) and seed number per pod (Fig. 5-c, d). Rapeseed is most susceptible to water stress over the flowering stage, during which pollen development can be restricted by water stress, and its effects lead to pod abortion by preventing flowers from developing into pods, as well as pod abscission (Ahmadi and Bahrani, 2009). Concurrently, the decline of the assimilate availability caused by drought shown in a great reduction in *A* (Fig. 3-e) could have had detrimental effects on the development of pods and in turn seeds during the seed filling stage (Istanbulluoglu et al., 2010). However, very few pods formed from rapeseed plants grown in 1-L pots may be primarily accounted for by the small size of pots as discussed earlier. Limited rooting volume could accelerate the severity of soil water deficit developed by withholding irrigation for WS plants as seen that soil VWC on two occasions approached PWP (−1.5 MPa) (Fig. 2-a, d), which could have resulted in a large quantity of pod abortion (Poorter et al., 2012).

Water stress at the critical stage, particularly at late flowering, has multiple impacts on the assimilate supply of pods and concurrently it also restricts the capacity of surviving pods and seeds for compensatory growth (Kirkegaard et al., 2018). In the current work, yield components showed different responses to AT across two experiments that make it difficult to interpret because of multiple variabilities from the individual plant, environmental conditions etc. However, we failed to identify statistically significant relationship between irrigation and AT in seed yield, although improvements (not significant) were observed from AT-treated WS plants. This implies that the compensation of yield components occurred following AT application during late flowering stage and/or seed filling stage by altering number of seeds and individual seed weight (Labra et al., 2017). Labra et al. (2017) stressed that rapeseed has the plasticity to adjust its potential seed number and size according to the assimilates produced at different stages. One possible hypothesis to explain the lack of effect of AT application, is that the concentration of AT may not be high enough to detect the yield benefits significantly on rapeseed due to the limitation of leaf coverage, which is highly related to the concentration of AT. The leaf coverage from AT application at 1% was estimated to be up to 19%, depending on the growth of plants (Xiang et al., 2021).

The duration, intensity and timing of water stress would affect crop yield (Müller et al., 2012). Those factors are also highly associated with the response of crops to AT application. Extremely severe or mild water stress may prevent the efficacy from AT application on rapeseed (Faralli et al., 2017; Faralli et al., 2017). The water stress in our experiments was imposed over a short timescale, and rapeseed plants would exhibit isohydric behaviors at gas-exchange level with tight control of leaf water potential when subjected to this type of water stress, although irreversible damage still can be caused by prolonged and/or intense water deficit (Bodner et al., 2015). Moreover, rapeseed can show different adaptations if water stress is imposed progressively (Ilami and Contour-Ansel, 1997). Future work should therefore compare different timescales and severities of drought imposition, and in addition, as abscisic acid plays a role in mitigating drought damage to crops following AT application (Mphande et al., 2021), it is worth assessing the efficacy of AT on crops in terms of the synergistic effects of hydraulic and hormone signals.

5. Conclusions

We showed that the range of water stress during the flowering stage, as indicated by soil matric potential ($-1 \sim -0.34$ MPa) across three experiments, depressed gas exchange of rapeseed plants. Consequently, water stress resulted in a substantial reduction in seed dry weight of rapeseed with the largest effects on pod number, compared to other two yield components (seed number per pod and thousand-seed weight). Application of film AT inhibited gas exchange of both well-watered and water-stressed plants in two out of three experiments. Furthermore, the magnitude of inhibition was linearly related to AT concentration that stomatal conductance decreased ~1.4 times faster than photosynthesis rate with a 1% increase in AT concentrations irrespective of irrigation. The increases induced by AT application in some yield components were observed at some concentrations, however, the compensating trade-off between pod number and seed number per pod resulted in the lack of significant improvement in seed yield from AT in water-stressed or well-watered plants. Therefore, the response of rapeseed treated with higher concentration (> 1%) of AT under both glasshouse and field conditions with different stress scenarios requires further investigation to identify an optimum dose rate and to understand the situations when AT has the highest efficacy to mitigate drought damage.

CRediT Author contributions

Jie Xiang: Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization, Funding acquisition. **Laura H. Vickers:** Methodology, Writing – review & editing, Supervision. **Martin C. Hare:** Methodology, Writing – review & editing, Supervision. **Peter S. Kettlewell:** Conceptualization, Methodology, Formal analysis, Writing – review & editing, supervision. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agwat.2022.107732.

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