Growth chamber and field evaluation of physiological factors of two watermelon genotypes

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ABSTRACT

Drought is a major threat to food security worldwide, and drought-tolerant plants are a convenient model to study the mechanisms underlying drought tolerance. Some of the studies on drought tolerance mechanisms have been performed under controlled environments, while others have been undertaken under natural field conditions; nevertheless, it is important to evaluate the similarities and differences between the results obtained in each case. In this study, the physiological responses of a drought-tolerant wildtype and a drought-susceptible watermelon cultivar were evaluated under both natural and artificial environments. Although different in intensity, the trend of physiological responses was similar in both environments. After five days of exposure to drought, the wildtype showed a sharper decline (80.91%) in CO_2 assimilation in the field than in a growth chamber (65.81%). The non-photochemical quenching (NPQ) parameters showed lower values in the cultivar than in the wildtype, regardless of growth environment; additionally, final NPQ values recorded for the wildtype were significantly higher in field plants than in those grown in a growth chamber. Regression analysis showed a highly significant correlation between NPQ and photosynthesis in the wildtype in both environments, while the relationships were non-significant in field-cultivated watermelon. These findings demonstrate that artificial conditions can be used to study the trends of plant responses to environmental stress, but results must be interpreted with caution.

1. Introduction

In various parts of the developing world, there is increasingly frequent water shortages resulting from changes in rainfall patterns and the concomitant increase in temperature due to climate change. Specifically, and mainly because of the increasing ambient temperature Southern Africa has been highlighted as a potential hotspot regarding climate change, with an increased frequency of drought incidence observed over the past few decades (Serdeczny et al., 2016; Hoegh-Guldberg et al., 2018), a phenomenon which is anticipated to have several negative effects on dryland crop production (Ramakutty et al., 2002; Sloat et al., 2020).

For global food production to keep pace with an ever-increasing world population in the context of this climatic shift, it is of paramount importance to attain an in-depth understanding of crop physiology under water deficit and high temperature conditions, as only such understanding will allow the development of improved agricultural technologies and superior crop varieties.

Plant biologists often use artificial environment facilities to study the physiological and molecular responses of plants to environmental stress, because such facilities allow a precise and uniform control over growth conditions. Although these controlled environment studies have been successful in unravelling the genetic and environmental factors and their interactions affecting plant responses, extrapolation of the knowledge attained, to natural field conditions, is still challenging. Indeed, various studies have demonstrated that the observations of plant performance in controlled environments often differ from those in the field (Limpens et al., 2012; Poorter et al., 2016). Furthermore, Limpens et al. (2012) emphasised that the differences observed between controlled and field environments are likely an indirect effect mediated by other environmental factors. Arguments regarding the validity of controlled environment facilities for research aiming to evaluate the effects of natural environmental conditions on plants, have been constantly questioned across research fields. For example, according to one such argument, the use of environmental facilities might lead to a cultural 'glass wall' between laboratory and field scientists Kohler (2002), which in turn might lead to differences in the interpretation of plant phenotypes Blum (2014).

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Watermelon is an important crop with a high demand worldwide; however, it is well known for its high susceptibility to drought stress (Dube et al., 2020; Wijesinghe et al., 2020). On the other hand, wild relatives of cultivated watermelon, which inhabit the Kalahari Desert, show strong tolerance to drought, as they grow well under the harsh conditions prevailing in this desert (Mujaju et al., 2011). Several factors reportedly account for the superior drought tolerance of wild watermelon, including the accumulation of reactive oxygen scavenger citrulline (Akashi et al., 2001; Kawasaki et al., 2000), a fast stomatal response (Sanda et al., 2011; Malambane et al., 2018), and a dynamic regulation of excess light-energy dissipation (Nanasato et al., 2010). However, all this experimental evidence has been generated under controlled environments, which poses a great challenge when attempts are made to apply the results in the field. Therefore, this study compared the performance of two watermelon genotypes under controlled (growth chamber) and natural (open field) conditions in order to evaluate the extent to which the results obtained in the controlled environment can be used to interpret field crop performance.

2. Materials and methods

2.1. Plant materials

Two types of watermelon were used in this study: (1) *Citrullus lanatus* (acc. No. 101117-1), a highly drought-tolerant wild genotype (Kawasaki et al., 2000) that is a natural inhabitant of the Kalahari Desert in Botswana and has been self-pollinated at least three times, and (2) the commercial watermelon (*Citrullus lanatus*) cv. Matsuribayashi-777 (Hagihara Farm, Nara, Japan). Seeds were soaked in water, kept at 30 °C overnight in the dark, and planted the following morning as described below.

2.2. Controlled environment experiment

Environmental conditions in the growth chamber were set as follows: light intensity at 800–1,000 μmol m^{-2} s^{-1} under a 16/8 h light/dark photoperiod, temperature at 30 °C, and relative humidity at 50% for the entire experimental period. Plants were grown in 2.5 L potting bags filled with potting soil (Nouka-No-Tsuchi, Kinki-Nousan, Sayou, Hyougo, Japan). The pots were laid in a completely randomised design, and each treatment was replicated thrice. After germination, seedlings were watered every other day at the onset of the light regime to maintain soil moisture near field capacity until the soil surface was flooded for approximately 2 s. A 1,000-fold diluted Hyponex nutrient solution (HYPONeX, Osaka, Japan) was applied once a week. Plants were further grown until they reached the 4th true leaf stage, and drought stress was imposed on half of the plants by withdrawing irrigation.

2.3. Field experiment

The two species were grown in an open field at the Botswana University of Agriculture and Natural Resources (BUAN), in Sebele, Gaborone, Botswana, (24° 33' S, 25° 54' E), at 994 m above sea level. The research site is in the South-eastern part of Botswana, which is characterised by a semi-arid climate with mean annual rainfall ranging from 230 to 500 mm. The soil type at the experimental site was sandy loam, classified as Typic Haplustalfs Machacha (2011), with a pH of 4.7, organic carbon content of 2.1%, cation exchange capacity (CEC) of 4.33 cmol kg^{-1}, and electrical conductivity (EC) of 2.5 cmol kg^{-1}. The study was conducted between February and March 2017. The experiment was set up in a split-plot, randomised, complete block design (RCBD), with irrigation regime as main plot and genotype as sub-plot. Each experimental unit was replicated thrice. In the field, the soil was basally dressed using a commercial fertiliser (NPK ratio of 2:3:2 (22%)) at 19.8 kg/ha N. After germination, plants were watered daily at dawn until the 4th true leaf stage, to maintain soil moisture near field capacity until the soil surface

underneath the plants was covered with water for approximately 2 s. Drought stress was imposed at the 4th leaf stage by withdrawing irrigation to half the experimental plants. Environmental conditions during the growing period in the field were recorded using a Yokogawa Weather Station dl850e/dl850ev scopecorder (Yokogawa electric corporation, Tokyo, Japan) fitted with a DT85 series 3 Datataker (CAS Dataloggers, Chesterland, Ohio, USA) stationed in the field. Environmental data are shown in Fig. 1.

2.4. Measurement of soil water content

For the field experiment, soil water content (SWC) was determined at a depth of 15 cm and at 5 cm from plants, using a soil moisture probe meter (MPM-160-B, ICT International, Armidale, Australia) previously calibrated according to manufacturer instructions. For the controlled environment experiment, gravimetric SWC was assessed as described by Malambane et al. (2018).

2.5. Measurement of photosynthetic and chlorophyll parameters

Leaf chlorophyll content was measured 3–4 h after sunrise in field plants, and 4 h after the onset of the light regime in growth chamber plants, using an SPAD-502plus meter (Konica Minolta, Tokyo, Japan). CO_2 assimilation and chlorophyll fluorescence were measured in the 3rd true leaves using an open gas exchange system LI6400XT photosynthesis meter (LI-COR Biosciences, Lincoln NE, USA). A 2 cm radius infrared gas analyser was used for all measurements, with a chamber temperature of 25 °C, a CO_2 flow rate of 400 μmol mol^{-1}, a photosynthetic photon flux density (PPFD) of 1,000 μmol m^{-2} s^{-1}, and a relative humidity of 50%. CO_2 assimilation was measured 4 h after sunrise in field plants and at the onset of the light regime in growth chamber plants. In contrast, chlorophyll fluorescence parameters of dark-adapted leaves were measured early in the morning before sunrise or before the onset of the light regime when plants had dark-adapted for at least 5 h. The response of net photosynthesis to internal leaf CO_2 concentration (*A/Ci* curve) was determined under a PPFD of 1,000 μmol m^{-2} s^{-1}; ambient CO_2 concentration in the cuvette was controlled with a CO_2 mixer across a series of 400, 200, 100, 100, 200, 400, 600, and 800 μmol mol^{-1}; measurements were made after a steady-state equilibrium was reached. Net photosynthesis values were plotted against the respective internal leaf CO_2 concentrations (*Ci*) to produce an *A/Ci* response curve at different time points during drought stress. For chlorophyll fluorescence, plants were dark-adapted for 30 min by switching off the light in the growth chamber or covering the leaves of field plants with aluminium foil. After dark adaptation, minimum fluorescence (F_0) and maximum fluorescence (F_m) were determined using a measuring light of approximately 1 μmol m^{-2} s^{-1} and a 0.8 s saturating pulse at 8,000 μmol photons m^{-2} s^{-1}.

2.6. Statistical analysis

Data shown are the mean values of three independent measurements. One-way analysis of variance (ANOVA) was used to analyse the results using the STATA statistical package, and the means were compared using the least significant difference (LSD) test. Linear regression models were used to determine the relationship between photosynthesis and fluorescence parameters. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Environmental conditions during the experiments

The average air temperature in the field was 21.9 ± 0.36 °C (Fig. 1) during water stress treatment, which was lower than the 30 °C temperature set in the growth chamber experiment for the entire duration of
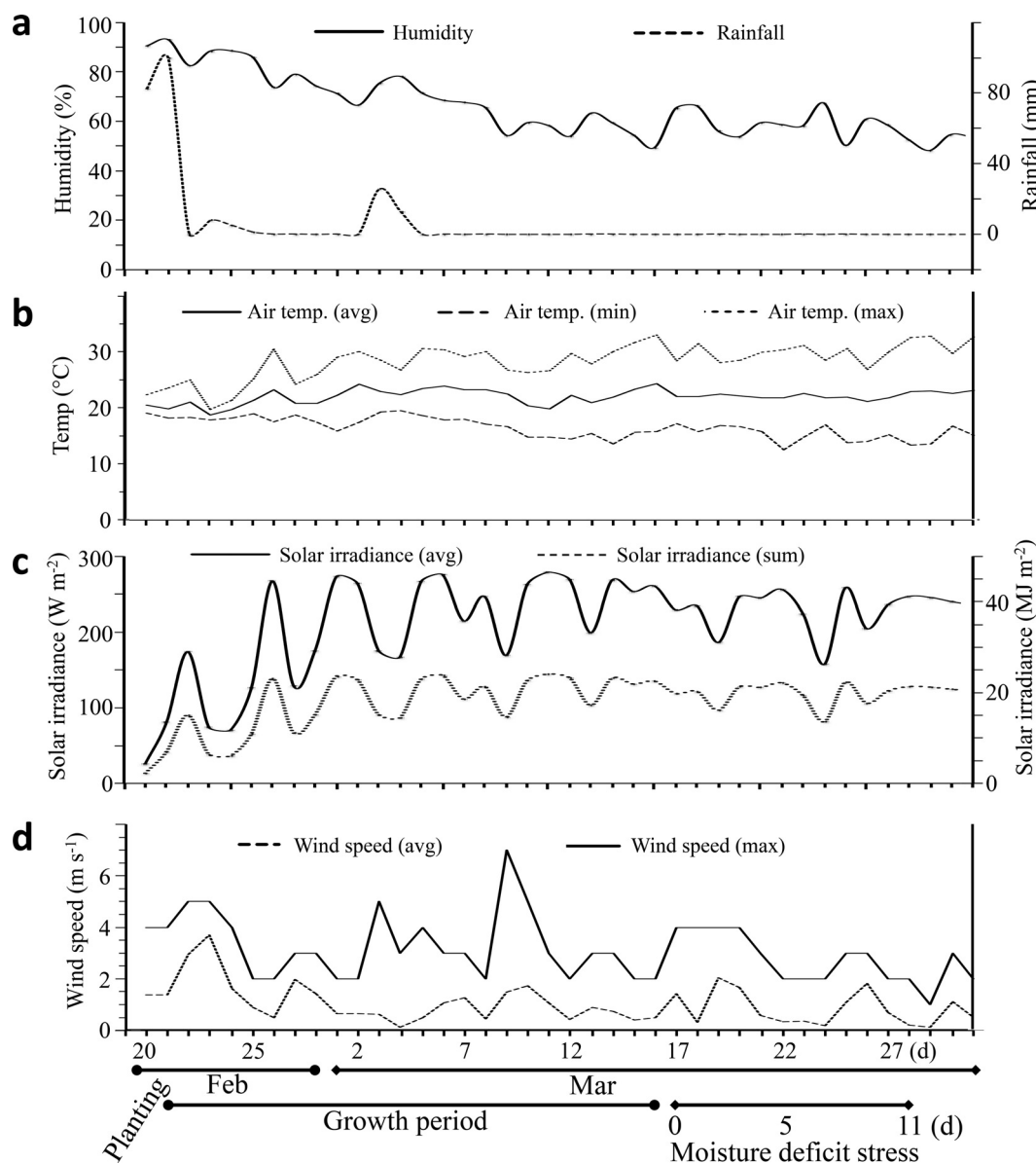
Fig. 1. Weather data during planting, monitoring, and data collection in the natural open field in Gaborone, Botswana. (a) minimum, maximum, and average daily temperature (°C); (b) daily precipitation (mm) and humidity (%); (c) solar radiation (W m−2 and MJ m−2), and (d) average and maximum wind speed (m s−1). The x-axis shows the study period in date-of-the-month format. DAI stands for days after stress induction.

plant growth. Temperature regimes differed markedly between the field and the growth chamber, as field conditions exhibited high variation, with minimum and maximum air temperatures being 15.4 ± 1.44 °C and 29.5 ± 1.38°°C, respectively. Additionally, rainfall during the water stress period was negligible, with an average of 0.1 ± 0.08 mm per day, and an average relative humidity of 59.5% ± 5.0%. Meanwhile, average solar irradiance recorded during the experimental drought period ranged from 200 to 250 W m^{-2}, while average wind speed recorded during the same period ranged from 0.5 to 2 m s^{-1}.

3.2. Changes in SWC and plant morphology

Plant roots are the first to experience moisture deficit and are the initiators of signal transduction pathways associated with the drought response mechanisms. Thus, SWC was measured in order to evaluate the magnitude of the limitation in soil water availability under both environments tested. The results showed a rapid decline in SWC at every sampling time point (DAI), thus underlining the difference in the

degree of stress over the experimental period under both growth conditions (Fig. 2). The initial soil moisture content, recorded on the final day of irrigation for both genotypes, was near 80% of the field capacity (FC), and the decline was observed as early as 3 DAI, reaching the wilting point at 9 DAI. The SWC recorded at 11 DAI showed a significant drop to 10.65% and 10.66% for wildtype and cultivated watermelon plots, respectively, in the field experiment. In contrast, in the controlled environment, SWC recorded at 11 DAI was 12.86% and 16.27% for the wildtype and cultivar, respectively.

3.3. Photosynthesis and leaf physiological parameters

Photosynthesis, an important process in plant growth, has been shown to be highly affected by drought. In this study, similar trends but varying degrees of changes were observed in photosynthetic rates in the tested genotypes in response to soil moisture deficit under both the experimental conditions (Fig. 3a). In the open field, the average photosynthesis rates (*A*) in irrigated plants were 27.63 ± 1.46 and
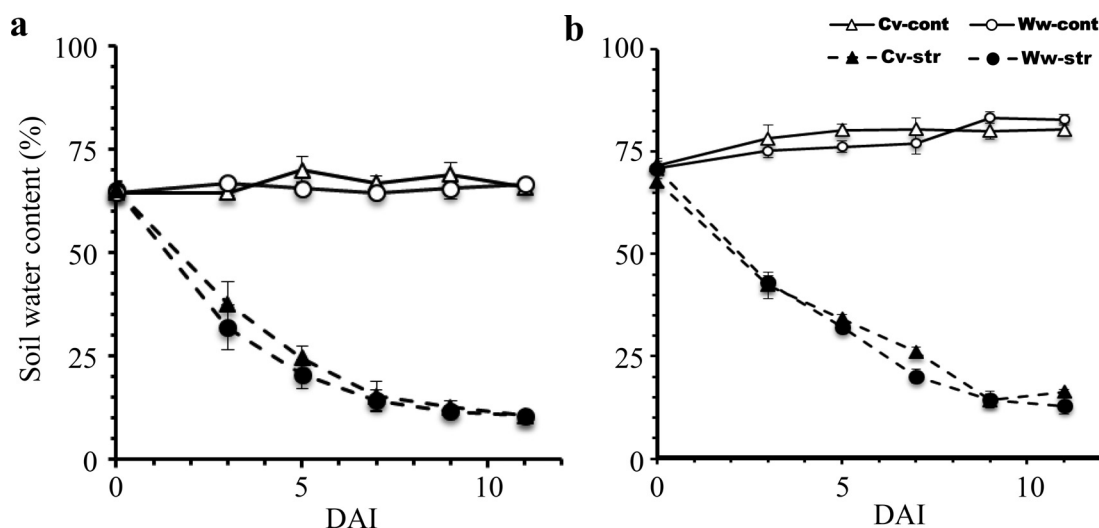
Fig. 2. Effects of drought stress on soil moisture content in a controlled environment (a), and in the field (b). Open and closed triangles (Cv-cont and Cv-stress) represent cultivated watermelon under control and drought stress, respectively, while open and closed circles (Ww-cont and Ww-stress) represent wild watermelon under control and stress conditions, respectively. Values are means ± SE (n = 3).

22.22 ± 0.46 μmol CO_2 m^{-2} s^{-1} for the wildtype and cultivar, respectively. In contrast, under the controlled environment, the average values were 24.35 ± 1.51 and 20.62 ± 1.08 μmol CO_2 m^{-2} s^{-1} for the wildtype and cultivar, respectively. The response of the wildtype to moisture deficit was slightly more pronounced in the field, with a drop to 4.76 ± 1.39 μmol CO_2 m^{-2} s^{-1} at 5 DAI, as compared to 8.24 ± 1.28 μmol CO_2 m^{-2} s^{-1} recorded at the same sampling time point in the controlled environment. A delayed response was observed for cultivated watermelon under both growth conditions; at 5 DAI, assimilation was reduced to 10.44 ± 1.71 and 12.25 ± 1.40 in the field and growth chamber plants, respectively. On the final day of data collection (11 DAI), the percent decline in photosynthesis rate in the wildtype was 97.5% and 97.4% for the field and controlled environment plants, respectively. On the other hand, the corresponding percent decrease for cultivated watermelon was 91.9% and 87.3% for the field and controlled environment plants, respectively.

The two watermelon types showed different kinetics of stomatal conductance and internal CO_2 concentration when exposed to moisture deficit (Fig. 3b,c). A rapid decline in conductance was observed in the wild watermelon, with a three- and a two-fold reduction at 3 DAI for the field and controlled environment plants, respectively. During 3–5 DAI, cultivated watermelon did not show any significant decrease compared to the significant response observed in the wildtype.

Both watermelon types showed a reduced chlorophyll content, as evaluated from SPAD readings, when exposed to drought stress, regardless of growth conditions (Fig. 3d). The decrease was sharper in field plants than in their growth chamber counterparts. At 11 DAI, the SPAD values for field-grown plants were 26.93 ± 4.37 and 14.73 ± 5.95 for the wildtype and cultivar, respectively, while the mean values for plants grown in the growth chamber were 36.43 ± 2.98 and 22.56 ± 1.49 for the wildtype and cultivar, respectively (Fig. 3d). The analysis showed that values remained relatively high in the wildtype as compared to those in the cultivated watermelon, indicating further differences within types when grown in two different environments.

3.4. Analyses of quantum yield of photosystems II (ϕPSII) and electron transport rate (ETR)

The average ϕPSII values for the two plant types under study were higher under field conditions than under the controlled environment, when provided with an unlimited water supply (Fig. 4a). However, a rapid decline was observed in ϕPSII values upon initiation of drought

stress, regardless of plant genotype or growth conditions. The wild genotype grown in the field showed a much greater decline than that in the cultivated genotype or the wild genotype grown in the growth chamber. A highly pronounced genotypic difference was observed in the field-grown plants, where the recorded ϕPSII values were 0.064 ± 0.026 and 0.17 ± 0.024 for the wild and cultivated genotypes, respectively, at 5 DAI. The same trend was observed throughout the duration of the moisture deficit treatment up to 11 DAI. A similar response pattern was observed for ETR for both genotypes under the two growth conditions (Fig. 4b).

Additionally, a decline in fluorescence parameters Fv/Fm and qP was observed with the progress of drought stress (Fig. 5a,b). Although the trend of decline was similar for the two environments tested, differences were observed in the magnitude of this decline. In the field, Fv/Fm values fell to approximately 0.72 for both genotypes, without any significant differences between genotypes. Conversely, a slight difference was observed between genotypes at 11 DAI in the controlled environment, in which case, the Fv/Fm values for wild and cultivated watermelon were 0.74 ± 0.003 and 0.73 ± 0.004, respectively.

In the wildtype, exposure to drought resulted in a massive increase in NPQ and qN (Fig. 5c,d), regardless of growth conditions. The values peaked at 7–9 DAI in the growth chamber, while in the field, the increase was not observed until 11 DAI. In contrast, lower increase in the NPQ and qN values was observed in cultivated watermelon.

3.5. A/Ci curve analysis

Maximum net photosynthesis rates (A) recorded at elevated CO_2 concentration in field-grown plants were 32.67 ± 2.89 and 21.62 ±€ 1.78 μmol m^{-2} s^{-1} for the wild and cultivated genotypes, respectively, at 0 DAI (Fig. 6a), while in the controlled environment, A was 26.7 ±€ 1.04 and 16.2 ±€ 1.38 μmol m^{-2} s^{-1} for the wildtype and cultivar, respectively (Fig. 6b). This maximum value was recorded at 800 μmol CO_2 mol^{-1} in both field and controlled environments.

The maximum A value for both genotypes under irrigation was within the same range for the period of data collection. When plants were exposed to drought stress, the A/Ci curve showed a significant downward shift under both the controlled environment and field conditions (Fig. 6a,b). The decline was more intense in plants grown under field conditions than in those grown under controlled environmental conditions. At 11 DAI, A values were high for plants of the cultivated genotype under both growing conditions, compared to those of the wild-
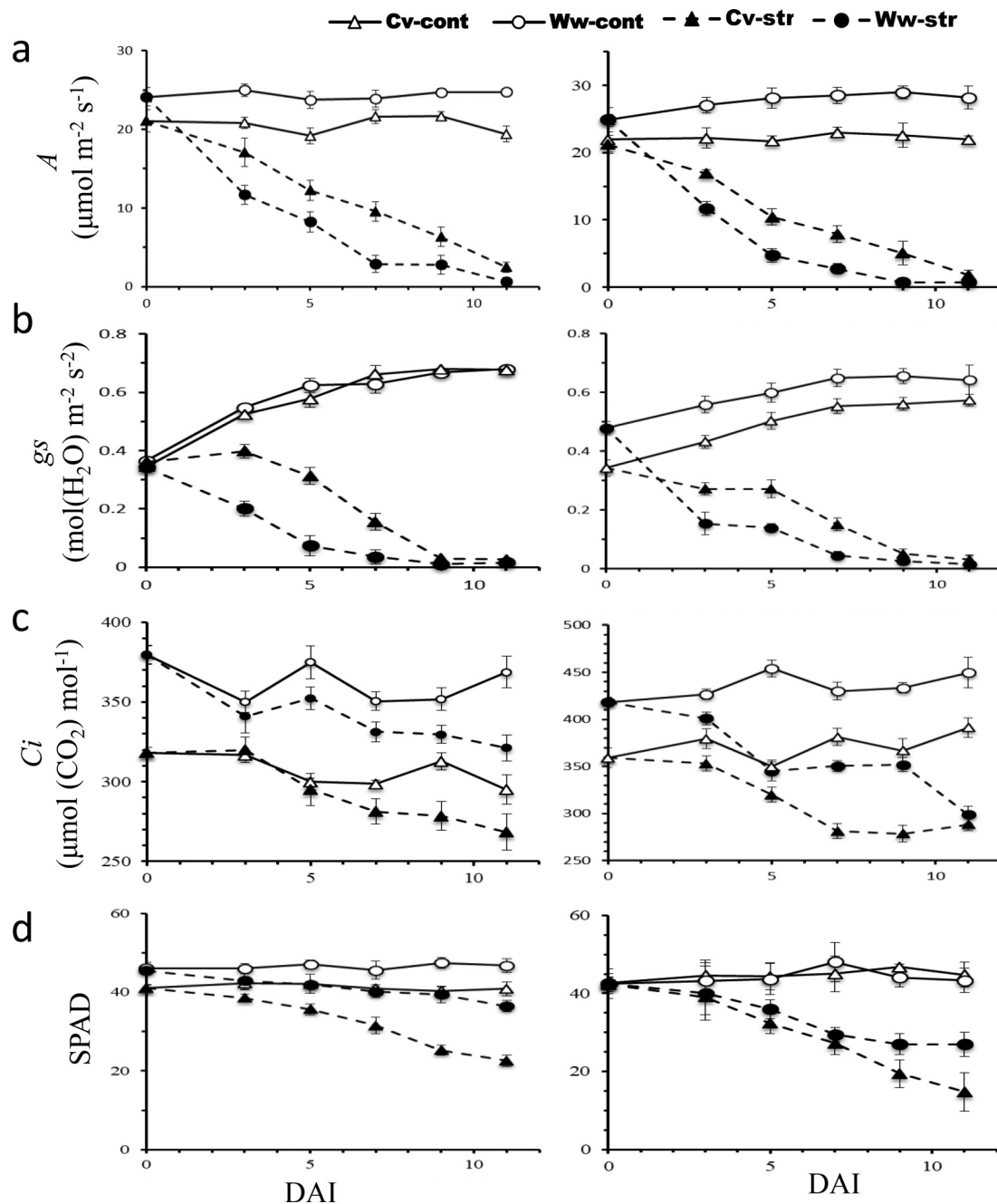
Fig. 3. Effects of soil drought stress on (a) photosynthetic rate (A), (b) stomatal conductance (gs), (c) intercellular CO_2 concentration (Ci), and (d) chlorophyll content (SPAD). Left panels represent the data collected from plants grown under a controlled environment, while the right panels show the data collected from those grown in the field. DAI indicates days after watering was withheld. The graph symbols conform to Fig. 2. Values are means ± SE ($n = 3$).

type plants. The cultivated genotype continued to show a response to CO_2 elevation even under extreme drought stress, as evident from its slightly higher photosynthesis rate.

3.6. Regression analysis

Linear regression analysis was performed to determine the correlation between the photosynthetic response and fluorescence parameters. In irrigated watermelon, the relationship between photosynthesis and fluorescence parameters showed mixed results, mostly non-significant for both the wildtype and cultivated watermelon (data not shown). In contrast, significant positive correlations were observed between most of the fluorescence parameters and photosynthetic rate in both the cul-

tivated and wildtype watermelon under both environments (Table 1). As for the wildtype, highly significant and negative correlations were observed between qN and NPQ responses and photosynthesis in both genotypes under the two environments.

The analysis of variance showed that genotype, environment, and their interaction, significantly influenced the variations in most parameters under evaluation. The effect of environmental conditions was significant for most measured parameters, except for Fv/Fm, conductance, qN, and internal CO_2, indicating that these parameters were less affected by the environment in which the two genotypes were grown.

Genotype significantly affected almost all measured parameters, except for ETR, suggesting that most of the variation experienced was determined by the difference between the two genotypes. The G × E effect
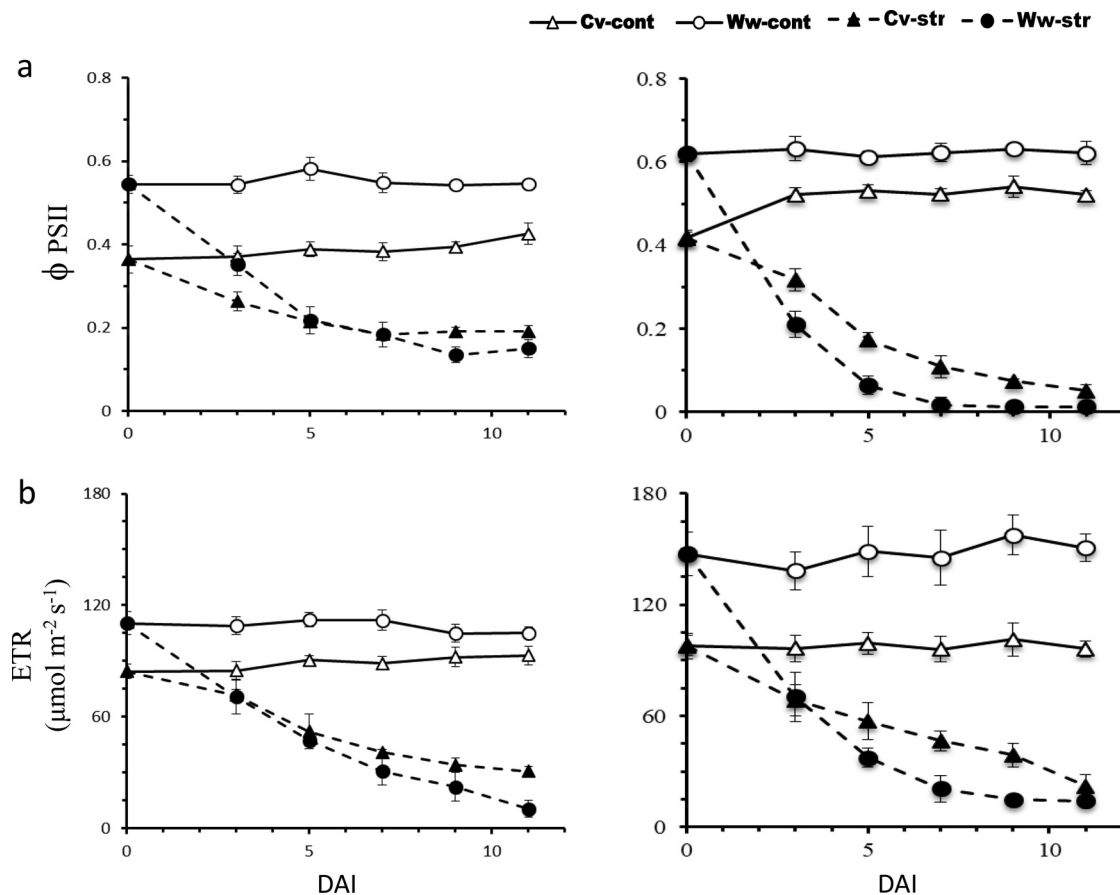
Fig. 4. Effects of moisture deficit on photosystem II efficiency (ΦPSII) and electron exchange rate of the PSII (ETR) in two watermelon genotypes. Left panel represents the data collected from plants grown in a growth chamber, while the right panel shows the data collected from field-grown plants. DAI stands for days after induction of drought stress. The graph symbols conform to Figure 2. Values are means ± SE ($n = 3$).

Table 1
Regression analysis of the fluorescence parameters against photosynthetic rate for two watermelon genotypes under field and growth-chamber environmental conditions.

| | Photosynthesis | | | |
| | Controlled environment | | Field environment | |
	Wildtype	Cultivated	Wildtype	Cultivated
A	1	1	1	1
Fv/Fm	0.966**	0.849*	0.984**	0.890*
ΦPSII	0.982**	0.878*	0.990**	0.988**
ETR	0.989**	0.981**	0.999**	0.981**
Cond	0.986**	0.954**	0.993**	0.911*
qP	0.980**	0.892*	0.968**	0.742 ns
qN	-0.95**	-0.843*	-0.958**	-0.857*
Chlo	0.923**	0.976**	0.891*	0.977**
NPQ	-0.924**	-0.921**	-0.976**	-0.730 ns
Ci	0.870*	0.910*	0.856*	0.871*

**, *, and ns indicate significant at <0.01, <0.05, and non-significant probability level, respectively. *A*, photosynthetic rate; Fv/Fm, ratio of fluorescence to maximum fluorescence; ΦPSII, photosystem II efficiency; ETR, electron transfer rate; Cond, conductance; qP, photochemical quenching; qN, qN parameter of non-photochemical quenching; Chlo, chlorophyll content; NPQ, NPQ parameter of non-photochemical quenching; *Ci*, internal carbon dioxide.

also showed significant differences for almost all the parameters, except for NPQ, which showed a non-significant interaction, thus indicating that the interaction effect depended on the differences between the two growth conditions.

4. Discussion

Field environmental conditions are highly variable and altogether exert strong and multiple impacts on plant physiology and morphology (Limpens et al., 2008). Indeed, at extreme levels, environmental factors limit plant growth, productivity, and survival (Dickinson et al., 2004; Guidi et al 2008; Otkin et al., 2013; Qaderi et al., 2019). One of the most detrimental environmental factors is soil moisture deficit, as it restricts the amount of soil water actually available to plants.

In this study, we set up two contrasting experimental environments, namely, a growth chamber where uniform growth conditions were precisely controlled, and an open field in the semi-arid climate of Botswana (Fig. 1). In these environments, two watermelon genotypes were either provided with an unlimited water supply or subjected to a water deficit by withdrawing irrigation. Monitoring soil moisture status is important for the formulation and testing of mechanistic hypotheses related to the mechanisms of plant drought tolerance or adaptive responses to moisture deficit Jones (2007). The decrease in soil water content during drought treatment showed a similar pattern under both environmental conditions regardless of genotype (Fig. 2), suggesting that stress intensity in terms of soil moisture availability was comparable under both experimental situations.

According to our results, the wildtype showed a faster response in reducing the CO_2 assimilation activity when exposed to drought stress than the commercial cultivar (Fig. 3a). The reduction in photosynthesis during drought stress has been widely reported in plants, as it has been observed that drought reduces foliar gas exchange Pinheiro and Chaves (2011). A previous study by Mo et al. (2015) on a drought-tolerant wild watermelon (genotype M20) and a susceptible culti-
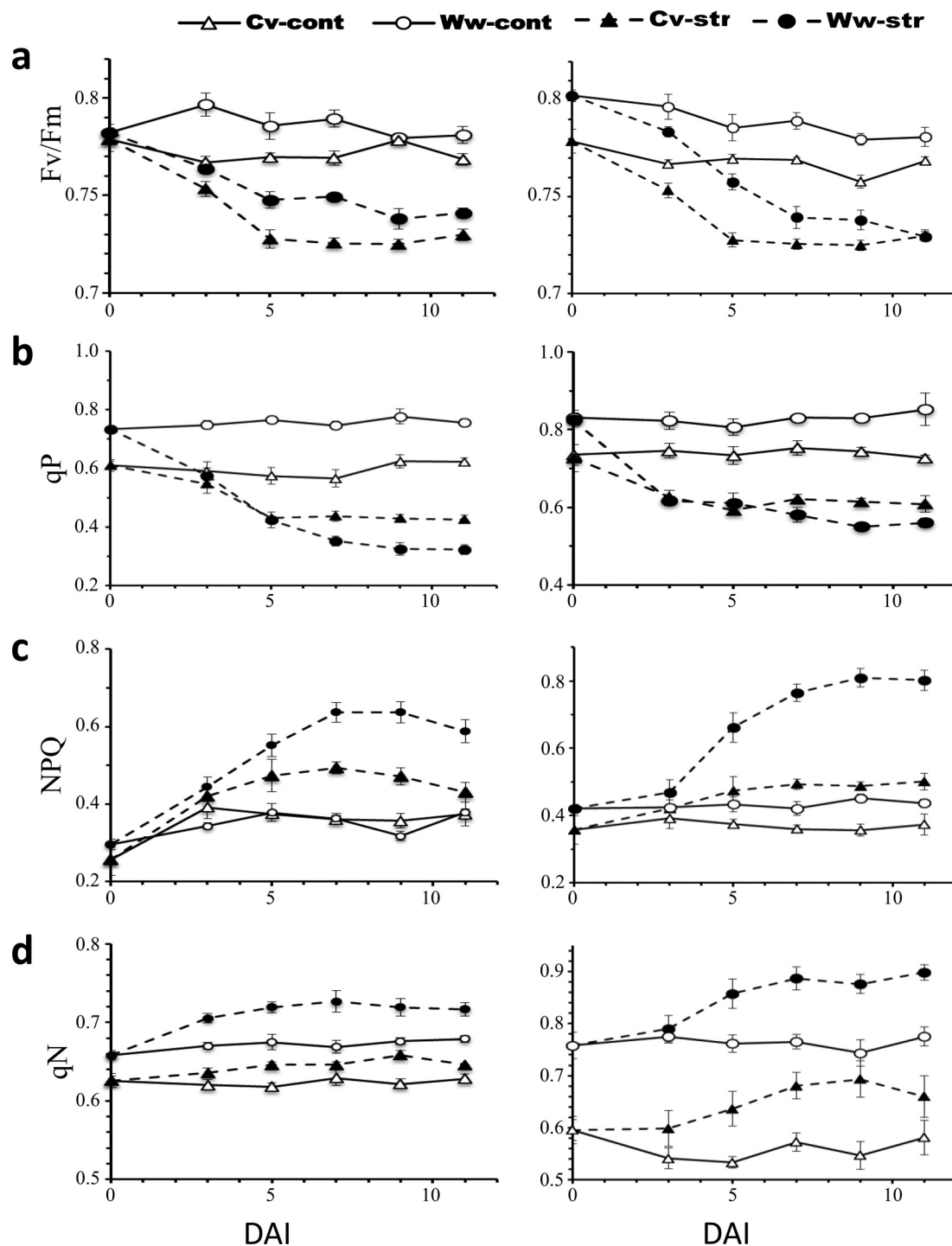
Fig. 5. Effects of moisture deficit on chlorophyll fluorescence parameters: (a) maximum quantum yield of PSII (Fv/Fm), (b) photochemical quenching (qP), (c) non-photochemical quenching (NPQ, $(F_m-F_m')/f_m'$), and (d) qN $((F_m-F_m')/F_m)$. The left panel shows data collected from plants grown in a growth chamber, while the right panel shows the data collected from field-grown plants. DAI stands for days after initiation of the water withholding treatment. The graph symbols conform to Fig. 2. Values are means $\pm$ SE ($n = 3$).

var (genotype Y34) reported a similar trend, suggesting that drought-tolerant genotypes react in the same manner and magnitude in response to drought stress. A contrasting response in the rate of photosynthesis rate was observed by Li et al. (2017), in which case, a drought-susceptible genotype showed a greater photosynthesis rate decline than the drought-tolerant genotype under drought stress.

Here, the wildtype closed its stomata faster than the cultivar, when exposed to drought stress (Fig. 3b). The drought-induced reduction of

CO_2 assimilation rate in most plants has been attributed to stomatal closure Pinheiro and Chaves (2011). Further, it has been suggested that stomata close quickly and sometimes completely when plants are exposed to drought stress (Athar and Ashraf, 2005; McAdam et al., 2015), although the rate of stomatal closure seemingly varies within species, with drought-tolerant genotypes having an advanced mechanism of closing their stomata (Pirasteh-Anosheh et al., 2016). Consistent with the findings of aforementioned studies, our results showed that the drought-
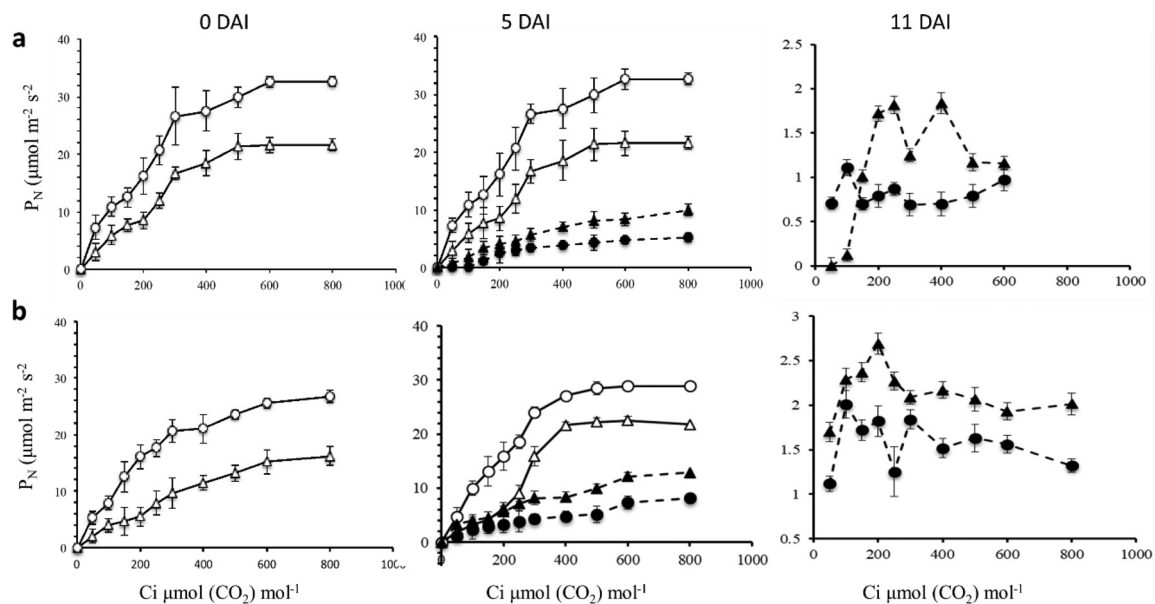
Fig. 6. *A/Ci* curves showing the effects of different CO_2 internal concentrations on watermelon plants exposed to drought stress. The top panel (a) shows the data collected from those grown under field conditions, while the lower panel (b) shows the data collected from plants grown under the controlled environmental conditions. DAI stands for days after drought treatment initiation. The graph symbols conform to Fig. 2. Values are means ± SE (*n* = 3).

tolerant wildtype responded faster to drought stress than the cultivar, by restricting stomatal opening sooner.

This study showed that the drought-susceptible watermelon cultivar had lower chlorophyll values than the tolerant wildtype (Fig. 3d). This might be related to the drought tolerance mechanisms of the plant, as the more tolerant genotypes have the capacity to protect oxidised chlorophyll molecules and have been used as physiological markers to estimate drought tolerance among genotypes (Turyagyenda et al., 2013; Feller et al., 2016; Czyczylo-Mysza and Myskow, 2017). The two different environments evaluated herein had a considerable effect on chlorophyll content, as field values were lower than those in the growth chamber (Fig. 3d). This observation was consistent with the findings reported by Li et al. (2018), suggesting that chlorophyll levels are influenced by climate; therefore, chlorophyll can be used as an indicator of how plants respond to climate change. Further, in both environments and both genotypes, chlorophyll content was highly correlated with photosynthesis (Fig. 3a,d, Table 1), indicating that the reduction of leaf chlorophyll content had a direct impact on photosynthetic performance. Gitelson and Metzlyak (2003) pointed out that it is important to determine whether the decline in photosynthetic rate is associated with damage to chlorophyll; the present study shows that there is a high correlation between the two. Similarly, other studies have demonstrated that drought stress-induced reduction in photosynthesis can be explained by damage to photosynthetic pigments (Efeoglu et al., 2009; Batra et al., 2014). Most importantly, the chlorophyll molecule is the light-harvesting component of the photosynthetic complex; thus, any effect on chlorophyll may potentially result in changes in the photosystem complex.

A different rate of reduction in the photochemical reaction of photosynthesis was observed in the wildtype and cultivar studied here (Fig. 4a,b). The ΦPSII response can be used as a drought tolerance indicator, as it is the most sensitive component of the photosynthetic system (Sharma et al., 2014; dos Santos et al., 2017). A rapid decline in ΦPSII under drought stress indicates that PSII activity has been downregulated to protect the photosynthetic machinery, as suggested by Liu et al. (2012). Therefore, a genotype that can quickly reduce its PSII activity has a better chance of survival under stressful conditions, as observed in our study for the wildtype, which reduced its PSII activity more quickly than the cultivar, thus mitigating the effects of drought.

ΦPSII is intimately linked to ETR Baker (2008); therefore, a reduction in ΦPSII should result in a concomitant reduction in ETR. Indeed, a high correlation between ETR and ΦPSII was confirmed in our study, in accordance with the results of Goltsev et al. (2012) and Batra et al. (2014), who showed that drought effects on the photosynthetic apparatus are associated with the effects of stress on the electron transport chain.

Non-photochemical quenching dissipates excess energy as heat, whereby it is believed to act as a photoprotective mechanism for survival under extreme environmental conditions (Zait and Schwart, 2018; Sachez-Reinoso, 2019). Consistently with this concept, here, we observed that the energy-dissipation capacity, as estimated from the NPQ and qN parameters, was higher in the wildtype than in the cultivated genotype (Fig. 5c,d), thus suggesting a better drought tolerance mechanism in wild watermelon than in the domesticated cultivar. Mishra et al. (2012) and Maxwell and Johnson (2000) proposed that NPQ can be potentially used for routine quantitative estimation of drought tolerance in plants.

Examination of the relationship between net CO_2 assimilation rate (*A*) and calculated internal CO_2 concentrations (*Ci*) through *A/Ci* curve analysis, has become an important tool to identify the specific factors responsible for limiting photosynthesis under drought stress (Manter et al., 2000; Manter and Kerrigan, 2004; Zhou et al., 2015; Kelly et al., 2016). Our results showed a strong downward shift in *A/Ci* curves under extended drought stress in both genotypes regardless of environmental conditions (Fig. 6a,b), indicating an impairment of photosynthetic capacity in water-stressed plants by a non-stomatal limitation, as suggested by Centritto et al. (2009). Variations between the two genotypes under study were observed for the corresponding *A/Ci* curves (Fig. 6a, b), which may be attributable to several internal (metabolic and/or genetic factors, among others) and/or external factors (environmental response, water/nutrient use efficiency, among other factors), as suggested by previous reports (Manter and Kerrigan, 2004; Dihn et al., 2017).

Regression analysis showed a strong correlation between chlorophyll fluorescence parameters and the photosynthesis rate (Table 1). Genotype, environment, and the interaction between the two had a significant impact on these physiological parameters (Table 2). Our observations are consistent with previous reports that plant physiological responses are influenced not only by the genetic and environmental compositions

Table 2

Analysis of variance with mean squares for the physiological and fluorescence parameters measured in two watermelon genotypes grown in the field or a growth chamber environment.

SOV	DF	A	Fv/Fm	ΦPSII	ETR	Cond	qN	qP	SPAD	Ci	NPQ
Environment	1	242**	0.02**	0.1**	109 ns	0.0 ns	0.0**	0.3**	18,600*	187,000	0.9**
Genotype	1	186**	0.01*	0.2**	9,070 ns	0.8**	1.2**	0.9**	203,000**	678,000**	5.9**
G × E	3	3,170**	0.01**	0.2**	111,000**	0.1**	0.1**	0.4**	13,400**	1,680,000**	0.0 ns

**, *, and ns indicate significant at <0.01, <0.05 and non-significant probability level, respectively. SOV: source of variance; DF: degrees of freedom; A: photosynthetic rate; Fv/Fm: ratio of fluorescence to maximum fluorescence; ΦPSII: photosystem II efficiency; ETR: electron transfer rate; Cond: conductance; qP: photochemical quenching; qN: qN parameter of non-photochemical quenching; SPAD, SPAD value for chlorophyll content; NPQ, NPQ parameter of non-photochemical quenching; Ci, internal carbon dioxide.

of the plant but also by the interactions between them (Hoeck et al., 2000; Miranda et al., 2009; El-soda et al., 2014; Genard et al., 2017).

5. Conclusion

The physiological responses of two watermelon genotypes to drought stress in an open field varied relative to their performance in a growth chamber in terms of magnitude, although the trend of change was similar in both genotypes. The wildtype showed a quicker response than the cultivated genotype for most physiological parameters measured under drought stress. Thus, the photosynthesis rate of wildtype dropped rapidly over the first few days of exposure to drought stress, while that of cultivated watermelon took a slightly longer time to drop, implying a slower response. In contrast, the dissipation of excess heat energy, an important process that mediates drought tolerance, was induced to a larger extent and showed a significant negative correlation with photosynthetic rate in the wildtype, whereas the more drought-susceptible cultivated watermelon recorded lower NPQ values and showed a weaker correlation with CO_2 assimilation, suggesting a lesser degree of drought tolerance and a lesser ability to protect the photosynthetic apparatus in the cultivated genotype. Analysis of variance suggested that not only genotype but the environment and their interaction also significantly influenced the variations in most physiological parameters measured, suggesting that, although they showed similar trends as those observed in the field in terms of physiological responses, the experimental results obtained in the controlled environment tested should be interpreted with caution when considering plant performance in the field .

Declaration of Competing Interest

None.

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