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Fusarium pseudograminearum infected wheat lines vary in disease severity and gas exchange response under different watering regimes

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Abstract

Crown rot (CR; *Fusarium pseudograminearum*) is a serious disease in winter cereals. Soil type, temperature, nutrients, water availability and stubble-borne inoculum levels play major roles in determining disease severity. This paper reports the impact of two different watering regimes on the disease severity and gas exchange of *F. pseudograminearum* infected bread wheat for the first time. *Fusarium pseudograminearum* inoculated and noninoculated genotypes with different susceptibility to CR were watered to either field capacity or a reduced watering regime in three controlled environment experiments. Rate of photosynthesis, stomatal conductance, internal CO_2 concentration and transpiration rate were measured using a portable photosynthesis system, together with disease severity of leaf sheaths at 28 days after planting. Significant differences in disease severity were reported between watering treatments with reduction in CR symptoms in the partially resistant genotypes in the reduced water treatment. Photosynthesis, stomatal conductance and transpiration rate were significantly decreased across most genotypes when inoculated with *F. pseudograminearum*. Differences in gas exchange between inoculum treatments were more evident in plants watered to field capacity. Water availability has been reported to be one of the crucial factors for initiating *F. pseudograminearum* infection and subsequent development of CR disease. This research demonstrates significant variation in genotype-related responses to the complex interactions of *F. pseudograminearum* infection and water treatment, with a negative impact of both limited soil water availability and CR disease severity on plant gas exchange in bread wheat.

KEYWORDS
bread wheat, crown rot, *Fusarium pseudograminearum*, photosynthesis, transpiration, water availability

1 | INTRODUCTION

Crown rot (CR) is a serious stubble-borne disease of wheat and barley, important in Australia and internationally (Kazan & Gardiner, 2018). CR was estimated to cause annual yield losses in Australia valued at AU$79 million and $18 million per annum, in wheat and barley, respectively (Murray & Brennan, 2009, 2010). CR is caused by a number of *Fusarium* species. Within Australia, these *Fusarium* species include *Fusarium culmorum*, *F. graminearum* and predominantly *F. pseudograminearum* (Fp) (Obanor & Chakraborty, 2014). In seedlings, the most characteristic symptoms are necrotic lesions on the coleoptile, which develop to a brown discolouration on the subcrown internode and leaf sheaths (Kazan & Gardiner, 2018; Percy et al., 2012). The infection cycle of Fp consists of three stages. In the first stage, Fp infects the plant and proliferates around the site of infection. In the second stage, termed the lag stage, a small increase in fungal biomass and symptom development is observed. The last stage is necrotrophic where the pathogen invades the internal stem crown tissue and causes the development of lesions and stem browning in the tillers of more mature plants (Beccari et al., 2011; Stephens et al., 2008). Both *F. graminearum* and Fp have been shown to behave as hemibiotrophic in the lag phase (Kazan et al., 2012).

Agricultural practices in Australia, such as minimum tillage used to improve soil moisture, have led to significant increases in the occurrence of CR (Kazan & Gardiner, 2018). Since the adoption of minimum or no till systems, control of CR is principally based on crop rotation to a nonhost or the use of partially resistant varieties, which over time decreases field inoculum (Wildermuth et al., 1997). To date, only partial levels of resistance to CR are available in cereal varieties (Kazan & Gardiner, 2018). Genetics studies have focused on screening wheat and barley populations to identify quantitative trait loci (QTLs) closely linked to resistance genes, leading to identification of 13 QTLs in the partially resistant hexaploid lines 2-49, Sunco, W21MMT70 and IRN479, which are each used in this study, and CPI 133814 (Bovill et al., 2006, 2010; Collard et al., 2005; Martin et al., 2015). QTLs on chromosome 1DL of 2-49 and IRN497 were identified at the seedling stage. However, QTLs on chromosomes 1AS, 1BS and 4BS in 2-49 bread wheat genotypes and on chromosome 2BS contributed by Sunco were observed at both the seedling stage and maturity in field trials. Bovill et al. (2006) identified three QTLs on chromosomes 2B, 2D and 5D in W21MMT70 at the seedling stage.

Soil type, temperature, water availability, nutrients and stubble-borne inoculum levels form complex relationships that play major roles in determining the severity of CR disease (Felton et al., 1998). Additionally, water availability is one of the most crucial factors for initiating CR infection and subsequent development of disease symptoms. Liddell and Burgess (1985) found that initial CR infection of wheat seedlings is favoured in moist soil when the water potential is between −0.3 and −0.7 MPa and that low infection occurs when the water potential is less than −1.5 MPa. In contrast, Beddis (1992) examined the relationship between soil water potential and seedling infection and observed that the incidence of infection was increased in both a tolerant and non-tolerant wheat variety grown under moisture stress or low water potential, which assists the pathogen to colonize seedlings. Further investigation is required to comprehensively understand the relationship between Fp infection, water availability and the subsequent development of disease symptoms in wheat varieties.

Plant–water relations can be directly altered and damaged by pathogen infection. The symptoms of infected plants can be similar to symptoms of water deficiency, for example, wilting. Previous physiological studies have indicated that *Fusarium* spp. are able to cause occlusions in the host xylem vessels (Walters, 2015). Only limited studies have been conducted on the infection process and host response of wheat to infection with Fp. Knight and Sutherland (2016) investigated Fp colonization in bread wheat, durum wheat and barley stems harvested at 10, 16 and 22 weeks after inoculation. During the infection process, all cell types, including vascular tissues, were colonized in stems of the cereals examined. Those authors suggested that blockage of vascular tissue and the subsequent restriction of water and nutrient translocation within the plant contributes to the reduction in grain yield and the establishment of white heads in susceptible genotypes. However, further information on the relationship between CR development and water stress across wheat varieties is required.

Both water deficiency and pathogen infection can induce a defensive response in the plant in order to maximize survival. The changes in water status may trigger hydrological signals via pressure volume changes in sensing cells or cause temporary cavitation in leaf veins, leading to increased stomatal conductance and water loss through transpiration (Christmann et al., 2013). Moreover, reports on water stressed wheat indicate direct relationships between water deficit and decreases in gas exchange parameters including photosynthesis (A), stomatal conductance (g_s), internal CO_2 concentration (C_i) and transpiration rate (E; Sharifi & Mohammadkhani, 2016; Zhao et al., 2020). Other reports have also indicated a reduction in gas exchange parameters due to fungal infection. Gas exchange parameters can vary depending on the growth development stage. In adult crops, leaves are the primary source and the roots, stems and grain are the sink, whereas in the seedling phase leaves can act as both the source and the sink (Yang & Luo, 2021). In the current research, we examine the disease severity and gas exchange response of wheat seedlings infected with Fp under different water regimes.

Understanding the physiological response of wheat genotypes with different levels of resistance to Fp is important for the future development of resistant and tolerant germplasm, enabling better management of CR disease. To the best of our knowledge, this is the first report of the effect of Fp infection on the gas exchange of bread wheat genotypes. We hypothesized that CR disease development is influenced by water availability. In addition, it was hypothesized that the wheat seedlings may vary in gas exchange response to both pathogen infection and watering regime.

2 | MATERIALS AND METHODS

2.1 | Plant material and experimental setup

To test the effect of water stress on the disease severity of six wheat genotypes inoculated with Fp, a series of seedling experiments were conducted in a controlled environment growth room at the Leslie Research Facility, Department of Agriculture and Fisheries, Toowoomba, Qld, Australia. Three replicated experiments were conducted in 2016 and in 2017 with six wheat genotypes that were selected according to their previously established susceptibility to CR (Table 1). Four treatments were applied to the six bread wheat genotypes consisting of a Fp-inoculated and a noninoculated pot each watered to either field capacity or reduced water (67% of field capacity). Each treatment by genotype combination was replicated three times forming a total of 72 pots as experimental units in each experiment; treatments were randomized to pots and arranged in the experiment following a randomized complete block design.

Soil used in this study was a self-mulching black Vertosol of the Irving clay soil association (Thompson & Beckmann, 1959), obtained from the Darling Downs, Qld, Australia. Soil was mixed with river sand (50% sand:50% soil) and heated to 80°C with a steam sterilizer for 40 min and air-dried for 7 days. No fertilizer was added to the mix. Plastic pots (7.5–9 cm diameter × 10 cm height = 500 cm^3) were filled with 295 g of dry soil and moistened to field capacity. The soil was levelled, and eight seeds of each genotype were lightly pushed into the surface. Seeds were covered by 160 g of sieved dry soil and the surface levelled. Then, 0.45 g of ground Fp inoculum was sprinkled over the soil surface of the inoculated pots and another 40 g of dry fine soil added to each pot. Fp inoculum consisted of sterilized wheat grain inoculated with a mixture of five aggressive isolates used in routine CR disease screening at the University of Southern Queensland (Percy et al., 2012). All pots were placed in the growth room set to 25°C/21°C in a 12 h day/night schedule and a light energy of 600 µmol m^{-2} s^{-1}. The inoculum was activated after 7 days by watering each pot to approximate field capacity or reduced water on a digital scale. Weights of 600 and 565 g were calculated to represent field capacity and reduced water (67% of field capacity), respectively. At this point, plants were thinned to five plants per pot. Pots were watered every 24 h to either field capacity or reduced water for the remainder of the experiment.

2.2 | Gas exchange and disease severity measurements

At 28 days after inoculum activation, gas exchange measurements were taken from plants in the controlled environment growth chamber using a portable photosynthesis system (LI-6400; LICOR). The measurements were taken from the first expanded leaf on two plants per pot. The temperature in the 6-cm^2 chamber was set at 25°C and the leaf level temperature was maintained at 1700 µmol m^{-2} s^{-1} using an in-built LED lamp (red/blue). Vapour pressure deficit was maintained between 1.9 and 2.1 kPa within this chamber during measurements. Each leaf was allowed 10–15 min to reach a steady state before measurements were taken. Rate of photosynthesis (A), stomatal conductance (g_s), internal CO_2 concentration (C_i) and transpiration rate (E) were recorded between 10:00 and 12:00 hours. Following gas exchange measurements, the plants were harvested, and excess soil was washed from roots and the lower crown region. After washing, plants were assessed for disease severity. Disease severity was measured on a 0%–100% rating scale of visual discolouration on the first four leaf sheaths of each of the five plants per pot. Rating of each tissue occurred in 5% increments where 0% is no discolouration and 100% is complete tissue discolouration.

2.3 | Data analysis

An across experiment analysis of data from the gas exchange parameters (photosynthesis, stomatal conductance, internal CO_2 concentration and transpiration rate) and disease severity assessments was conducted using a linear mixed model. A separate analysis was conducted for each trait, with the two groups of traits (gas exchange and disease severity) requiring different modelling approaches according to the structure of the experimental material measured. Additionally, as the assumptions of normality and homoscedasticity of the residuals were not fulfilled for disease severity, this trait was transformed using a square root transformation.

TABLE 1 Crown rot susceptibility of each wheat line and cultivar as defined in the published literature and 2014 Queensland wheat variety guide.

Genotype	Crown rot response	Reference
2-49	Partial resistance	Collard et al. (2005)
W21MMT70	Partial resistance	Bovill et al. (2006)
IRN497	Partial resistance	Wildermuth et al. (2001)
Sunco	Moderately susceptible	GRDC and DAFF (2014)
EGA Gregory	Susceptible	GRDC and DAFF (2014)
Livingston	Susceptible–very susceptible	GRDC and DAFF (2014)

Abbreviations: GRDC, Grains Research and Development Corporation; DAFF, Queensland Department of Agriculture, Fisheries and Forestry.

A single pot in each experiment formed the experimental unit, and five plants per pot formed the observational units. While gas exchange traits were measured on the first expanded leaf of an individual plant, namely one value per observational unit, disease severity was measured from the first four leaf sheaths of each plant, thus four values from the same observational unit. The models fitted to each trait included experiment, genotype, inoculum and water treatment, along with their respective interactions, as fixed effects. Additionally, for the disease severity trait, a fixed effect of leaf was also included, along with all resulting interactions with the previously described terms. Terms describing the structure of the experimental material (replicate blocks, pots, plants) were included as random effects in both models, with additional terms for leaf sheaths included in the disease severity model. Heterogeneity of residual variance was modelled between experiments and, where significant, the complexity of the residual variance structure was extended to allow for heterogeneous residual variance between inoculum treatment groups within and between experiments. Furthermore, for disease severity, an unstructured covariance model was considered to account for residual correlation between leaves of the same plant.

Models were fitted using the ASReml-R package (Butler et al., 2017) in the R statistical computing environment (R Development Core Team, 2020) with variance components estimated using residual maximum likelihood (Patterson & Thompson, 1971). The significance of fixed effects was tested using a Wald conditional test, while the significance of increasingly complex residual variance models was tested using a log-likelihood ratio test. All significance testing was performed at the 5% level.

3 | RESULTS

3.1 | Effect of water stress on disease severity of wheat genotypes infected with *F. pseudograminearum*

Disease severity was assessed for each plant based on the visual appearance of brown/black lesions from infection on the first four leaf sheaths of each wheat genotype seedling. During disease rating of IRN479, a dark purple colour was observed, which was difficult to distinguish from typical CR visual discolouration. For this reason, it is expected the disease severity measurements in IRN497 may be overestimated.

There was a significant Water × Inoculum × Genotype water interaction for disease severity ($p = 0.003$) in the leaf sheaths measured at 28 days after planting (Table 2). Average visual discolouration across the leaf sheaths was up to 60% in the Fp-inoculated treatments watered to field capacity, with W21MMT70 and 2-49 significantly lower than the other genotypes (Figure 1a). The average visual discolouration of the leaf sheaths in Sunco, IRN497 and 2-49 was significantly lower in the reduced water treatment compared to the field capacity treatment, while differences in disease severity between watering regimes were not significant in Livingston, EGA Gregory and W21MMT70 (Figure 1a). Visual discolouration of the leaf sheaths was low in the noninoculated treatments under both field capacity and reduced water treatments (<1.5%) of all genotypes (Figure 1b).

TABLE 2 Significant effects and interactions for disease severity and rate of photosynthesis (*A*), stomatal conductance to water vapour (g_s), internal CO_2 concentration (C_i), and transpiration rate (*E*) of inoculated and noninoculated bread wheat seedlings watered to field capacity or reduced watering.

Variable	Source	*p*
Disease severity	Water × Inoculum × Genotype	0.003
	Experiment × Inoculum × Genotype	0.002
A	Genotype × Inoculum	<0.0001
	Inoculum × Water	<0.0001
g_s	Genotype × Inoculum × Water	0.024
C_i	Genotype × Inoculum × Water	0.020
E	Experiment × Inoculum	0.006
	Genotype × Inoculum × Water	0.032

A significant Experiment × Inoculum × Genotype interaction for disease severity ($p = 0.002$) was also detected (Table 2), which indicated the variation between experiments in the average visual discolouration observed across genotypes. In Experiment 2, lower levels of disease were recorded in the inoculated treatments of several genotypes than were recorded in Experiments 1 and 3 (Figure 2a). The ranking of genotypes also varied between experiments. For example, the highest disease severity recorded in Experiments 1 and 2 was for EGA Gregory, while this cultivar was ranked in the middle range of disease severities in Experiment 3. The average visual discolouration of the leaf sheaths was low in the noninoculated treatments with a maximum of 4% disease severity recorded in EGA Gregory in Experiment 1 (Figure 2b).

3.2 | Leaf gas exchange parameters

Significant Genotype × Inoculum ($p < 0.0001$) and Inoculum × Water ($p < 0.0001$) interactions were reported for the average of rate of photosynthesis (*A*) (Table 2). The value of *A* in the noninoculated treatments was higher in the partially resistant genotypes (IRN497, W21MMT70 and 2-49) compared to the susceptible genotypes (EGA Gregory and Livingston) and the moderately susceptible genotype Sunco. Conversely, only slight differences were observed in *A* between genotypes inoculated with Fp, with the average *A* significantly lower in the Fp-inoculated treatments compared to the noninoculated treatments across all genotypes (Figure 3a). Overall, the average *A* value in the reduced water treatments was significantly lower than the treatments watered to field capacity in both the Fp-inoculated and noninoculated treatments, with the lowest *A* reported in the inoculated reduced water treatments (Figure 3b).

A significant Genotype × Water × Inoculum interaction was recorded for the values of stomatal conductance (g_s) ($p = 0.024$), C_i ($p = 0.020$) and *E* ($p = 0.032$) (Table 2). In the noninoculated treatments,

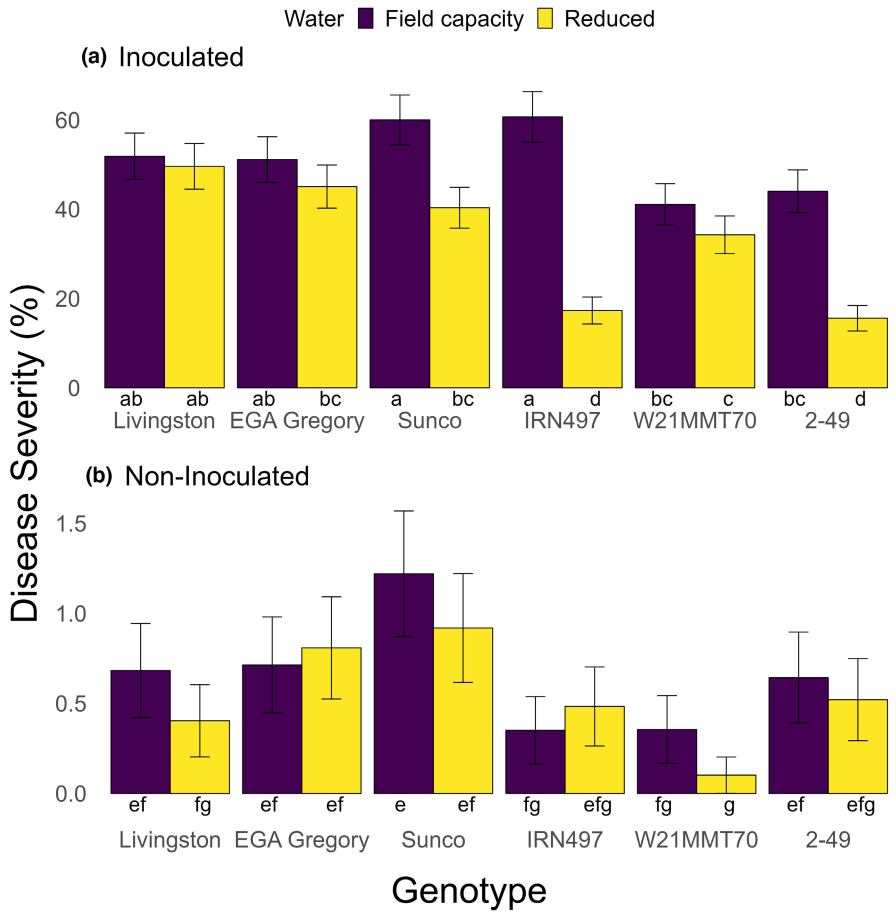

FIGURE 1 Disease severity of (a) *Fusarium pseudograminearum* inoculated and (b) noninoculated bread wheat seedling leaf sheaths, watered to either field capacity or reduced watering (67% of field capacity). Plants were assessed 28 days after planting and results averaged across three experiments. Data = mean ± *SE*; $n = 45$. Different letters represent values that are significantly different between genotypes, inoculum and water treatments ($p = 0.0015$).

all genotypes watered to field capacity recorded higher values of g_s compared to the reduced water treatment, with the exception of cv. Livingston, which had low g_s in both inoculated and noninoculated treatments under both watering regimes (Figure 4a). Inoculation with Fp significantly reduced g_s in all genotypes watered to field capacity (except Livingston) and only in EGA Gregory and Sunco in the reduced water treatments. EGA Gregory watered to field capacity without inoculation recorded the highest g_s value (0.32 mol^{-2} s^{-1}) followed by Sunco with the same treatments (0.28 mol^{-2} s^{-1}) (Figure 4a). The lowest g_s value (of 0.13 mol^{-2} s^{-1}) was recorded in EGA Gregory inoculated with Fp in the reduced water treatment.

The value of internal CO_2 concentration (C_i) was significantly higher in treatments watered to field capacity compared to the reduced water regime in both Fp-inoculated and noninoculated genotypes, with the exception of Livingston and Sunco in the noninoculated treatments (Figure 4b). Significantly higher C_i values were recorded in the inoculated genotypes compared to the noninoculated genotypes, with the exception of Sunco and EGA Gregory in the reduced water treatment and 2-49 under both water treatments (Figure 4b).

The value of transpiration rate (E) was significantly lower in plants with the reduced water treatment compared to those watered to field capacity in all genotypes in both the inoculated and noninoculated treatments, with the exception of Fp-inoculated Livingston. Inoculation with Fp also reduced the value of E irrespective of water regime (Figure 4c). Transpiration rate was highest for W21MMT70 (4.6 mmol H_2O m^{-2} s^{-1}) followed by 2-49 (4.1 mmol H_2O m^{-2} s^{-1}) and EGA Gregory (3.6 mmol H_2O m^{-2} s^{-1}) when watered to field capacity without inoculation. The lowest E value was recorded in EGA Gregory (1.1 mmol H_2O m^{-2} s^{-1}) grown under reduced water and Fp inoculation. A significant interaction between experiment and inoculum ($p = 0.006$) was also observed for E where the value of E was highest in Experiment 3 and lowest in Experiment 1 for noninoculated genotypes and highest in Experiment 1 and lowest in Experiment 3 for the Fp-inoculated genotypes (data not shown).

4 | DISCUSSION

In the last 20 years, CR research in Australia has concentrated on identifying and integrating genetic resistance into commercial cultivars, disease evaluation methodologies, and production loss in infected wheat and barley (Forknall et al., 2019; Hollaway et al., 2013; Kelly et al., 2021; Percy et al., 2012). Limited information is available on how CR disease develops and how the host responds to infection in relation to soil water availability. In this study, we demonstrate the negative impact of limited soil water availability and CR infection on plant gas exchange in seedlings of six bread wheat genotypes with varied susceptibility to Fp in an environmentally controlled growth chamber.

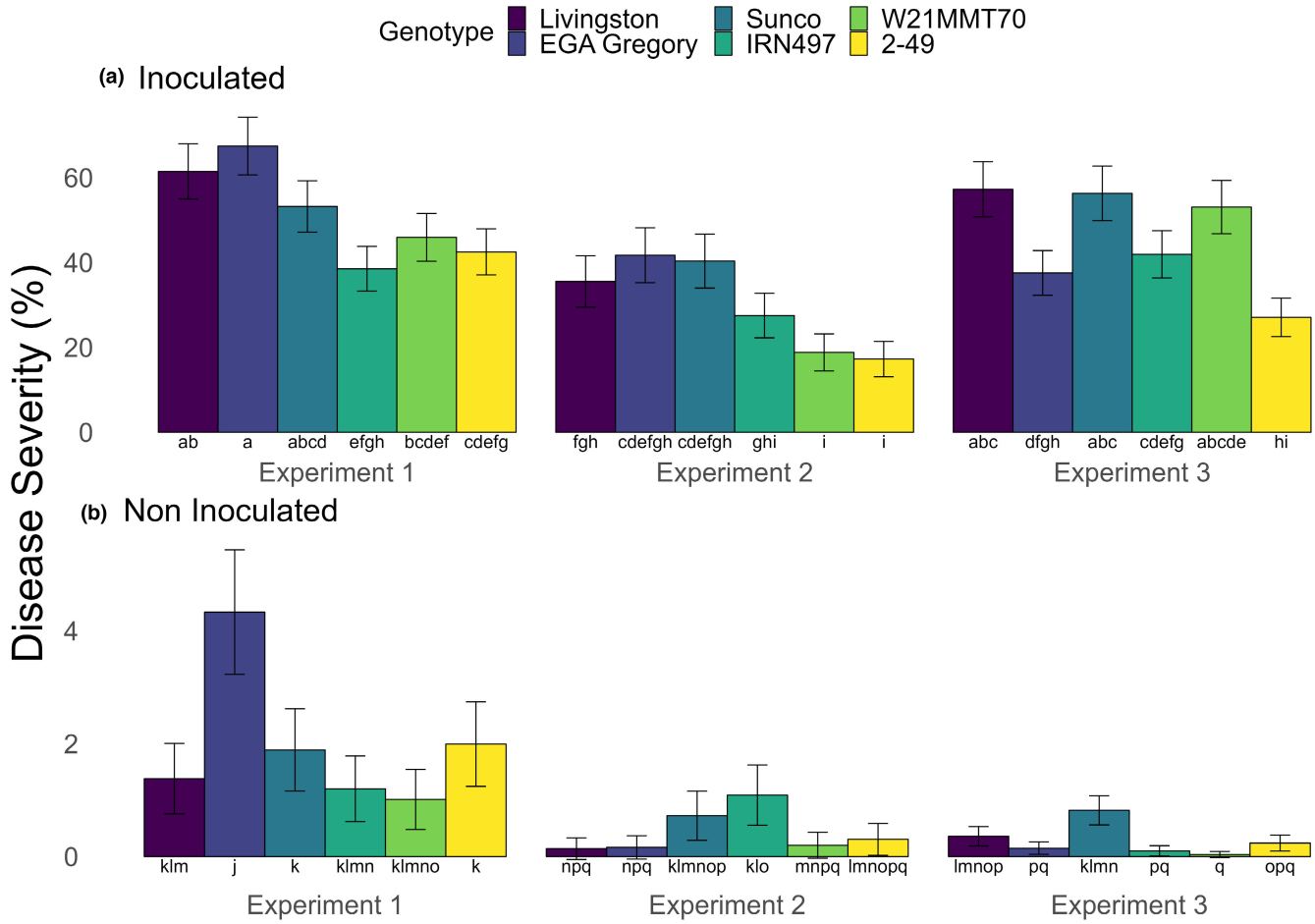

FIGURE 2 Disease severity of (a) *Fusarium pseudograminearum* inoculated and (b) noninoculated bread wheat seedling leaf sheaths, assessed 28 days after planting for each of the three experiments averaged across the water treatments. Data = mean ± *SE*; $n = 30$. Different letters represent values that are significantly different between genotypes, experiments and inoculum treatments ($p = 0.0015$).

This study provides evidence that Fp-inoculated wheat seedlings watered to field capacity produced higher visual discolouration on the leaf sheaths than when grown under a reduced watering regime. This is not consistent with previous reports demonstrating that water stress can enhance the CR proliferation in seedlings in controlled environment conditions (Beddis, 1992; Li et al., 2008). The water stress achieved in those two studies is likely to be more severe than the reduced watering treatment imposed in our experiments, where differences in disease severity between the water treatments were only significant in the partially resistant lines 2-49 and IRN497 and the moderately susceptible cv. Sunco. Our result indicates that partial resistance in seedlings is more pronounced when water is limiting. Ma, Du, et al. (2015) identified the QTL *Qheb.mda-3B* on chromosome 3B of wheat, which can control the content of malondialdehyde, a product of lipid peroxidation used as a parameter to assess the cellular damage of plants attributed to water stress. It was argued that such QTLs can form a strong association with drought tolerance located in the same region as *Qcrs.cpi-3B*, which was previously identified to control the resistance to *Fusarium* CR infection in wheat. A recent study by Buster et al. (2022) reported yield reductions in bread and durum wheat varieties in both field and controlled environment conditions, even when water was not limited, suggesting that the negative effects of Fp infection on yield are not just restricted to situations where water is limiting.

Photosynthesis is ultimately a substantial factor in plant development, and it is very sensitive to water deficits (Sharma et al., 2020). In our study, gas exchange measurements have been collected at the seedling stage, where plants can act as both the source and sink for photosynthesis, due to the accumulation of photosynthates that are required for growth and development (Yang & Luo, 2021).

Water stress was shown to strongly impact the gas exchange parameters in this study, significantly reducing the photosynthetic capacity of all genotypes. This result is in agreement with previous studies on wheat genotypes that demonstrated a reduction in *A* during water stress treatments (Ashraf & Harris, 2013; Chaves & Oliveira, 2004; Liu et al., 2016; Ma, Duan, et al., 2015; Sharifi & Mohammadkhani, 2016; Wu & Bao, 2011; Zhao et al., 2020). Other studies on plant physiology have also shown that drought stress can cause changes in the photosynthesis process, with Sun et al. (2013) identifying it as one of the first processes to be impacted. When

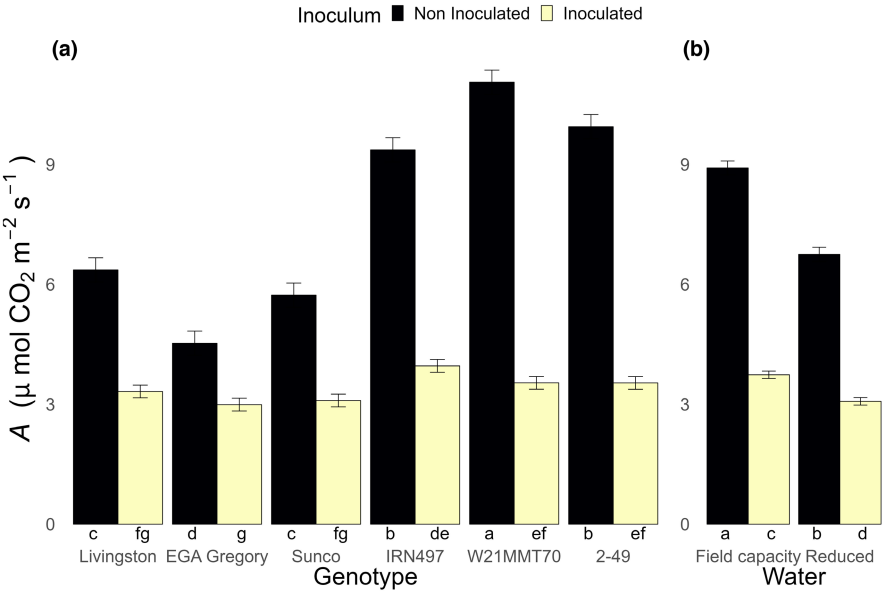

FIGURE 3 Rate of photosynthesis (a) in *Fusarium pseudograminearum* inoculated and noninoculated bread wheat seedlings, assessed 28 days after planting for (a) six genotypes with varying crown rot resistance averaged across experiments and water treatments, and (b) plants watered to either field capacity or reduced watering (67% of field capacity) averaged across genotypes and the three experiments. (a) Data = mean ± *SE*; $n = 36$. Different letters represent values that are significantly different between genotypes and inoculum treatments ($p = 0.0001$). (b) Data = mean ± *SE*; $n = 90$. Different letters represent values that are significantly different between water and inoculum treatments ($p = 0.0001$).

plants are exposed to drought stress, one of the first responses is to reduce transpiration by closing the stomata. Stomata regulate the exchange of CO_2 and water in plants, and their closure helps to limit water loss. However, this also leads to a decrease in CO_2 absorption and the transportation of nonstructural carbon (NSC), which is crucial for photosynthesis, and can lead to carbon starvation that affects various other plant processes (McDowell & Sevanto, 2010; Sevanto, 2014). Carbon starvation can cause stunted growth and negatively impact respiration under mild to moderate water deficit conditions (Pinheiro & Chaves, 2011).

In this study, in the noninoculated genotypes, the plants watered to field capacity had higher g_s, C_i and E than the plants in the reduced water treatments. These findings are in line with previous studies by Ma, Duan, et al. (2015), Saeidi and Abdoli (2015) and Zhao et al. (2020), where water deficit of wheat was reported to cause stomatal closure leading to a decreased availability of CO_2. Water stress decreases turgor pressure within the cell, and stomata react by partial closing to limit the transpiration to prevent excessive water losses, which in turn leads to a decrease in A, C_i and E (Thapa et al., 2018).

This is the first report of changes in gas exchange parameters of wheat seedlings varying in their response to CR infection, grown under reduced and field capacity water treatments. Our results demonstrate a strong negative impact on A across all wheat genotypes infected with Fp, reducing A in inoculated genotypes by approximately 60% and 70% for plants watered to field capacity and in the reduced water treatment, respectively. Thus, the lowest level of A was observed in the inoculated genotypes under the reduced water treatment. Yang et al. (2016) reported a reduction in A in Fusarium head blight (FHB)-resistant genotypes after *F. graminearum* inoculation but insignificant changes in the A parameters in FHB-susceptible genotypes. However, in our study, all genotypes (from susceptible to partially resistant) demonstrated significant reductions in A when inoculated with Fp, although the differences in A between the inoculated and noninoculated treatments were more pronounced in the partially resistant genotypes IRN497, W21MMT70 and 2-49. Hu et al. (2020) demonstrated the downregulation of photosynthesis-related genes in two wheat lines L658 (susceptible) and L958 (resistant) infected by the biotrophic powdery mildew fungus *Blumeria graminis* f. sp. *tritici*. This decrease in A was reported to be most likely related to inhibition of peroxidase (POD) and catalase (CAT), which regulate hydrogen peroxide (H_2O_2).

Fp generally infects the lower crown region, including the subcrown internodes, leaf sheaths and stem tissues. Although photosynthetic measurements were taken on the leaf blade tissue without disease symptoms, the A activity in the infected plants was found to be lower than in the noninoculated controls. Bastiaans (1991) and Debona et al. (2014) also reported a reduction in the A activity in the noninfected area in wheat during early infection by the blast pathogen *Pyricularia oryaze*. This reduction in A resulted from a toxin produced by the fungus that had diffused into the surrounding tissue, causing tissue disintegration, which compromised water uptake as well as phototranslocation in leaves far from the infection site. The relationship between photosynthesis and mycotoxin production in Fp CR infections requires further investigation.

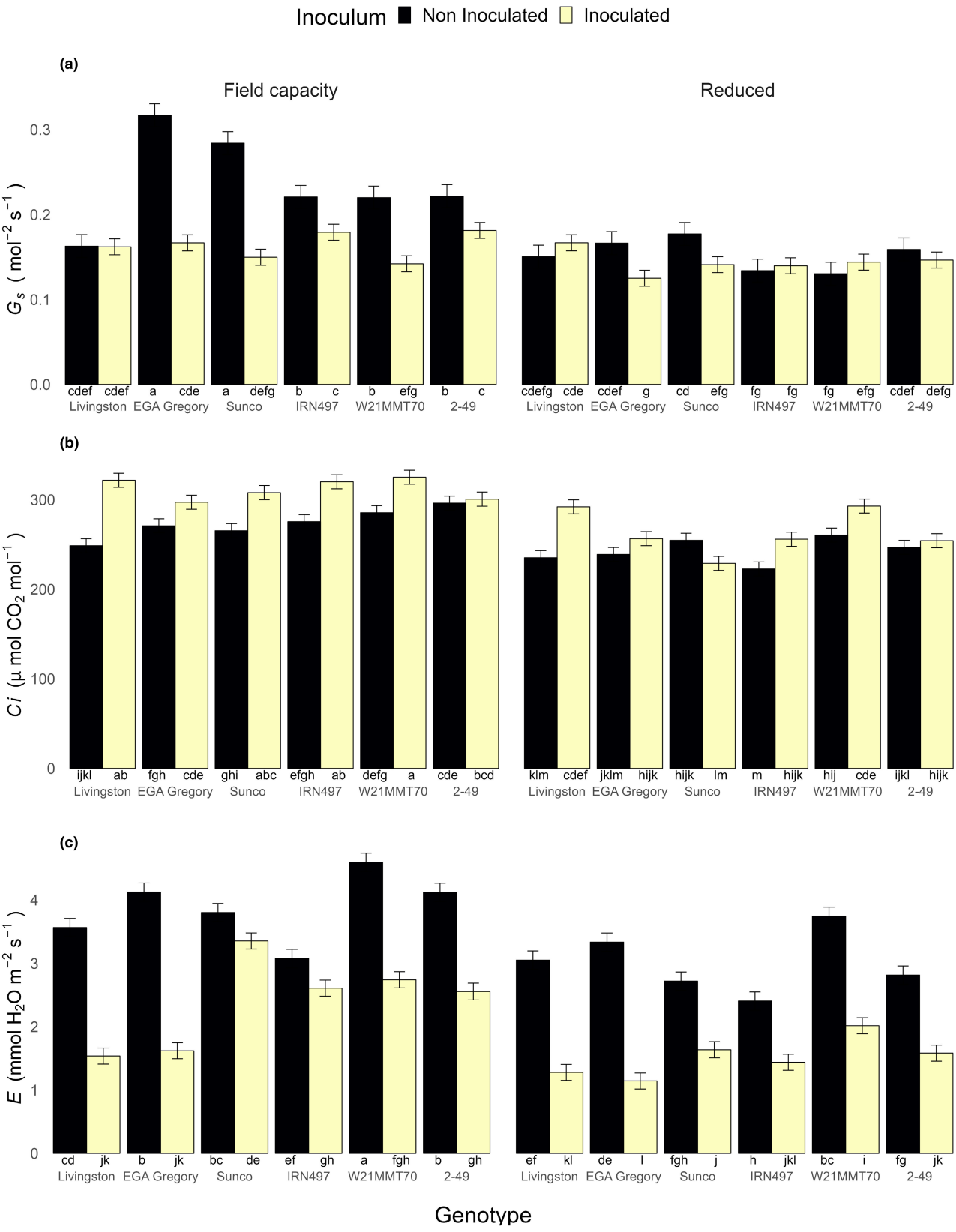

FIGURE 4 (a) Stomatal conductance (G_s), (b) internal CO_2 concentration (C_i), and (c) transpiration rate (E) of *Fusarium pseudograminearum* inoculated and noninoculated bread wheat seedlings, watered to either field capacity or reduced watering (67% of field capacity). Plants were assessed 28 days after planting and results averaged across three experiments. Data = mean ± *SE*; $n = 18$. Different letters represent values that are significantly different between genotypes, inoculum and water treatments ($p = 0.024$, 0.020 and 0.006 for a, b and c, respectively).

All genotypes inoculated with Fp recorded a significantly lower value of g_s, compared to the noninoculated genotypes, when watered to field capacity, with the exception of the susceptible cv. Livingston. Genotypes that were inoculated also exhibited higher C_i than the noninoculated plants in both the watering treatments with the exception of cv. Sunco. These data are in line with Tatagiba et al. (2016), who demonstrated a reduction in *A* with decreased g_s and increased C_i of rice leaves infected with *Monographella albescens*. Similar results have also been reported in other pathosystem investigations, and these findings have usually been interpreted as an indication of biochemical, rather than diffusive, limitations to photosynthesis (Dallagnol et al., 2011; Resende et al., 2012). Furthermore, high C_i values at advanced stages of fungal infection suggest that there are biochemical limitations that restrict CO_2 influx into the carboxylation sites on chloroplasts, due to a reduction in RuBisCO activity (Aucique-Pérez et al., 2017).

Wheat infected with fungal species have been reported to display a reduction in *A* activity due to poor RuBisCO performance, low inflow of CO_2 from the atmosphere to carboxylation sites in the leaf tissue and an increase in *E* (Debona et al., 2014; Rios et al., 2017). In addition, reduced *A* was observed in several biotrophic fungal infections including wheat infected with *Pyricularia* (Perez et al., 2014), and wheat infected by *Bipolaris sorokiniana* (Rios et al., 2017). The reduction in *A* during these infections was claimed to be linked to the non-stomatal factors such as decreased mesophyll conductance. Additionally, these fungal infections have been observed to alter the dissipation of light energy in wheat plants, with decreases in photochemical processes and increases in non-photochemical mechanisms. This leads to a decrease in photosynthetic pigments, such as β-carotene, xanthophylls, total chlorophylls and lutein, on the leaves of infected wheat plants.

The average *E* of the wheat genotypes in this study was lower in the Fp-infected plants than in the noninoculated controls in both watering treatments. Debona et al. (2014) observed lower *E* in wheat infected with *P. oryaze* compared to the controls. The observed lower *E* was ascribed to the control by g_s. Our current findings agree well with the work of Bermúdez-Cardona et al. (2015) where lower *E* was argued to be associated with the stomatal closure of leaf maize infected with *Stenocarpella macrospora* in order to avoid the excessive water loss. Silveira et al. (2019) also suggested that maize infected with *Exserohilum turcicum* can produce a reduced *E* compared to the noninoculated plants due to wilting leaves at the early stage of fungal infection. While in our study *E* was significantly reduced in all inoculated genotypes grown under both watering treatments, variation in *E* was observed. The highest level of *E* in the inoculated treatments was recorded in the moderately susceptible cv. Sunco, followed by the partially resistant lines IRN497, 2-49 and W21MMT70, when watered to field capacity. Additionally, the damage induced by Fp hyphae colonizing the vascular tissue may result in a reduction in the level of *E* in infected genotypes. Knight and Sutherland (2016) reported significant Fp hyphal colonization of vascular bundles that was expected to cause the vascular system in wheat plants to become dysfunctional. Additionally, the blocking of vascular tissue in susceptible genotypes may disrupt hydraulic conductivity to a greater extent than in partially resistant genotypes.

Gas exchange parameters are associated with each other and, consistent with our observation on this host pathogen interaction, a lower rate of *A* may be linked with a reduction in g_s and increase in C_i (Bermúdez-Cardona et al., 2015; Rios et al., 2017). In this case, the *A* reduction might have resulted from limited CO_2 fixation at the biochemical level or reduction in CO_2 influx due to stomatal closure (Debona et al., 2014). The observed gas exchange parameters among the six wheat genotypes included in this study indicated significant variation in genotype-related responses to the complex interactions of Fp infection and water treatment. It is possible that Fp may have been able to interfere with the wheat primary metabolism including amino acid, hormonal signals and organic acid for support and extension of fungal infection, and certain changes were observed among cultivars regardless of their level of resistance to CR. Further research on the biochemical and physiological response of wheat genotypes infected with Fp will improve our understanding of the mechanisms of resistance and tolerance to CR and therefore enable better selection of improved CR-resistant and -tolerant wheat varieties for integration into the Australian and international wheat breeding programmes.

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CONFLICT OF INTEREST STATEMENT

The authors have no competing interests to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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