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Abstract

Kiwifruit is a widely cultivated and economically important fruit crop. Italy is one of the largest producers of kiwifruit in the world and its industry plays an important role both nationally and internationally. In recent years, however, kiwifruit plantations and total production in several countries around the world, including Italy, have been threatened by a complex vine physiological decline syndrome triggered by soil water excess and unsuitable conditions. The current climate change scenario has serious implications for crop production and is certainly a new challenge for kiwifruit cultivation in many areas, urgently requiring more sustainable agricultural practices and adaptation strategies.

Kiwifruit is known to be extremely sensitive to climate and irrigation levels, which affect the viability of kiwifruit growing areas. A low physiological tolerance to drought and waterlogging makes the soil water environment important for healthy growth and optimum production of kiwifruit vines.

This thesis then focuses on evaluating the physiological responses of yellow-fleshed kiwifruit to water stress (both deficit and excess), thereby increasing the knowledge useful for implementing more precise irrigation strategies for this crop. Overall, the aims of this thesis were i) to comprehensively describe the physiological responses of yellow-fleshed kiwifruit vines to water stress, which is increasingly occurring due to climate change and improper irrigation management, ii) to implement more precise irrigation for such a sensitive crop in a very challenging environment (also due to the expected impacts of climate change), and iii) to evaluate the tolerance/susceptibility of different kiwifruit rootstocks to water stress in order to identify more tolerant genotypes to abiotic stress in kiwifruit.

First, a field experiment was conducted to evaluate the physiological behavior of yellow-fleshed kiwifruit vines under varying soil water availability in a Mediterranean environment. This study showed that kiwifruit can cope with challenging Mediterranean environmental conditions by precisely managing irrigation, distributing the right amount of irrigation water to meet the vines' water needs at the right time and place. Drought stress was imposed to study the physiological responses of the vines, through the analysis of the diurnal trends of stem water potential and stomatal conductance, identifying the onset of stress. Daily dynamics of soil water availability reduction and root uptake highlighted the importance of water availability in the top soil layers to provide the amount of water needed by the vines. Soil moisture thresholds were identified to provide the most suitable soil water environment and guide irrigation decisions. In addition, plant

measurements (i.e., sap flow and trunk water potential) were used in combination with a mechanistic plant model to characterize the hydraulic behavior of kiwifruit vines under different soil water availability conditions (i.e., well-watered and drought stress). As plant variables are monitored continuously, the modelling approach provides valuable information on the hydraulic functioning of vines under field conditions and throughout the season, and shows great potential for the development of irrigation decision support tools.

In a further step, drought and waterlogging induced responses of different kiwifruit rootstocks and scion/rootstock combinations were investigated. The differential response of the investigated genotypes was evaluated through an integrated physiological and phenotyping approach. In particular, image analysis was performed at the ALSIA 'Metapontum Agrobios' research centre (PhenoLab platform), which is part of the European Plant Phenotyping Network and infrastructures. Physiological and image-based phenotyping assessment provided an effective methodology to conduct a screening for more tolerant kiwifruit genotypes to water stress. The 'Bounty' rootstock was identified as the least susceptible to water stress and 'Hayward' and 'D1' as the most susceptible.

The results obtained will hopefully open the way to further studies that will support kiwifruit production in Italy and help to face new challenges in its cultivation.

1. General introduction

1.1 Climate change and water stress

Climate change is expected to increase the frequency and intensity of abiotic stresses to which plants will be exposed in many regions of the world (Onyekachi et al., 2019; Eckardt et al., 2023). The most obvious effects, such as warmer temperatures, more frequent extreme heat events, increased drought, salinity and flooding, are having a serious impact on agricultural production, threatening global food security (Naglaa et al., 2014; Pérez-Lucas et al., 2024). However, the magnitude and significance of the impacts of climate change on agricultural systems depend on local conditions. Mediterranean climate regions, characterized by mild winters and warm and dry summers, are vulnerable to climate change, with increasing droughts and high temperatures, water deficits and changes in precipitation patterns (Giorgi and Lionello 2008; del Pozo et al., 2019; Pérez-Lucas et al., 2024). The Mediterranean basin has been identified as one of the most prominent climate response ‘Hot Spots’ (Giorgi, 2006). The combined occurrence of extreme climatic hazards, such as heatwaves and droughts (i.e., concurrent hot and dry events, CHDs), is becoming more evident, threatening the stability of ecosystems and increasing the vulnerability of the Mediterranean region (Gazol and Camarero, 2022; Santos et al., 2024). Crop productivity is expected to decline in the near future due to adverse environmental impacts, increasing the need for agricultural adaptation, especially in Mediterranean climate regions, to improve crop performance and resilience to environmental stresses (Pérez-Lucas et al., 2024).

Drought events caused by low precipitation and increased evapotranspiration (Bouabdelli et al., 2022) are a worrying consequence of climate change, altering crop production, plant ecophysiology and the geographical distribution of crops (Eckardt et al., 2023). Considering future changes in temperature and precipitation, drought events in the Mediterranean basin are expected to become more severe and prolonged (Tramblay et al. 2020; Bouabdelli et al., 2022). Higher evapotranspiration rates combined with reduced precipitation lead to increased water requirements in warmer climates, with negative water balances and more frequent water deficits (Cardell et al., 2020).

On the other hand, an increase in heavy precipitation events due to global climate change have serious implications for the risk of waterlogging and flooding (Donat et al., 2016; Blöschl et al., 2017; Furtak and Wolińska 2023), also affecting some areas of the Mediterranean basin (Dayan et al., 2015; Tramblay and Somot, 2018). Mollema et al. (2012) carried out a water budget analysis in Northern Italy under future climate scenarios, showing an increase in summer water deficit and an increase in winter water surplus, explained by a relatively larger proportion of winter

precipitation. Waterlogging stress has recently become a major threat to plants, affecting their growth, development and productivity (Sharma et al., 2021; Manghwar et al., 2024; Topali et al., 2024). Overall, climate change is disrupting natural water cycles, exacerbating both flooding and water scarcity (Sarma et al., 2024).

In this scenario, effective adaptation strategies can be used to ensure more sustainable agricultural practices, reduce the negative impacts of water stress and increase crop resilience to a changing climate. Drought and flooding problems can be mitigated through appropriate soil and water management (Terán et al., 2024). Improving drainage and optimising irrigation can reduce the frequency and severity of waterlogging stress (Manghwar et al., 2024), while different irrigation strategies have been developed to save water resources and increase water use efficiency without causing significant yield losses (Terán et al., 2024). The implementation of water-efficient irrigation strategies and management is a key adaptation strategy (Pérez-Lucas et al., 2024), especially in the Mediterranean region, due to the expected increase in crop evaporative demands (Cardell et al., 2020) and regional water scarcity (Claro et al., 2024). Among adaptation strategies, breeding programs can provide climate-resilient cultivars with higher tolerance to different abiotic stresses, such as water stress (Manghwar et al., 2024; Terán et al., 2024). Research and development of climate-resilient cultivars with lower water requirements, improved water-use efficiency and adaptation to water-limited conditions and high temperatures (i.e., warmer climate conditions) (Cardell et al., 2020; Pérez-Lucas et al., 2024) or increased tolerance to waterlogging stress (Manghwar et al., 2024) is increasingly needed.

1.2 Kiwifruit overview and current challenges

Kiwifruit is a relatively new but economically and nutritionally important fruit crop cultivated in many different regions of the world (Ferguson, 1984; Pinto and Vilela, 2018; Sharma et al., 2020; Gao et al., 2022; Hazarika et al., 2022), including both northern and southern hemispheres (Mian et al., 2022). The most important kiwifruit species are native to China, where wild resources are still more important (Ferguson, 1984; Huang and Ferguson, 2007; Wang et al., 2018). However, New Zealand was the first country to exploit its commercial production and introduce it to the global market, becoming a major exporter of the fruit (Ferguson, 1984; Huang, 2016; Sharma et al., 2020; Hazarika et al., 2022). The global distribution and cultivation of kiwifruit began to increase as suitable areas were identified and farmers became more aware of the plant's requirements, allowing the industry to grow rapidly (Ferguson et al., 2023). In particular, the global kiwifruit production has been characterized by incremental growth in the 1970s, exponential growth in the 1980s, static production in the 1990s and steady growth in subsequent

years (Ward and Carol, 2013). Over the past 20 years, the global kiwifruit sector has continued to experience significant growth, both in terms of increasing supply, with the volume of the total fresh fruit category increasing by around 54% between 2000 and 2020, and in terms of increasing value of the fruit traded, which increased more than four-fold from 2000 to 2020 (Chamberlain, 2023).

China is currently the largest kiwifruit producer, followed by New Zealand and Italy, which is third (first in Europe) (FAOSTAT, 2023). According to FAO data, Italy ranked second in total kiwifruit production in 2017 and before, ahead of New Zealand, with its ranking having changed in recent years. In recent years, Europe has experienced variable weather conditions that have affected the cultivation and production of kiwifruit in Italy, which has also been challenged by the ongoing physiological decline (known as 'Kiwifruit Vine Decline Syndrome' (KVDS) or 'Moria') (Tacconi et al., 2015; Bardi, 2020) and management of the bacterial disease caused by *Pseudomonas syringae* pv. *actinidiae* (Psa) (Donati et al., 2014). In particular, Italian kiwifruit production declined notably from approximately 522 thousand tons in 2020 to around 391 thousand tons in 2023, with a corresponding decrease in the total cultivated area from 26,690 ha to 25,319 ha in 2023 (ISTAT, 2024).

More than 30 years ago, the kiwifruit industry was based on a single internationally grown and traded cultivar, 'Hayward' (*A. chinensis* var. *deliciosa*), which was recognized as the most popular, successful and important commercially marketed cultivar (Ferguson, 1999; Testolin and Ferguson, 2009; Ferguson, 2013; Pinto and Vilela, 2018; Costa et al., 2018). In recent years, however, the need for new cultivars has been recognized and several breeding programmes have been initiated, with priority given to yellow and some red-fleshed kiwifruit cultivars (Testolin and Ferguson, 2009; Costa et al., 2018). The supremacy of the 'Hayward' cultivar has therefore begun to be challenged by the growth of the kiwifruit industry and increasing consumer interest in new cultivars available from breeding programmes that claim superior characteristics to 'Hayward' (Asadi et al., 2024). In particular, yellow-fleshed kiwifruit cultivars have only recently (i.e., 2000) been introduced to the world market and were the first major products of formal kiwifruit breeding programmes (Costa et al., 2018). By 2022, two-thirds of kiwifruit exported from New Zealand was yellow-fleshed, which was also becoming increasingly important in other countries such as Italy, where it accounted for 15% and 25% of kiwifruit plantings and total production in 2020 (Ferguson et al., 2023).

Native kiwifruit habitats are characterized by high humidity, abundant precipitation, a sufficiently long frost-free growing season (225 to 240 days and 750 to 800 chilling units) and moderate light intensity (Ferguson et al., 1984; Smith and Walton, 2000). Most *Actinidia* species grow best in

relatively humid and sheltered environments with fairly cold winters and warm summers (with temperature not exceeding 40 °C) (i.e., warm, moist environments) (Ferguson, 2013). However, due to the different geographical distribution of the most commercially important and widely cultivated *Actinidia* species (*A. chinensis* var. *chinensis* from eastern and southern China and *A. chinensis* var. *deliciosa* from western China and higher altitudes (Smith and Walton, 2000; Huang and Ferguson, 2001; Ferguson, 2013)), *A. chinensis* var. *chinensis* is not quite cold hardy (Strick and Cahn, 1998) and has a lower chilling requirement compared to *A. chinensis* var. *deliciosa* (Wang et al., 2017), demonstrating its suitability for distribution in warmer locations.

The mapping of agroclimatic zones is a well-established method for delineating the most suitable areas for a particular crop (Salinger and Kenny, 1995; Gao et al., 2022). For kiwifruit, three major climatic factors have long been identified as important determinants of kiwifruit distribution: winter chilling, seasonal thermal time and rainfall requirements (Salinger and Kenny, 1995). However, the total area of suitability is increased if irrigation is available, so in areas that are thermally suitable but limited by low rainfall, the deficit could be met by irrigation (Salinger and Kenny, 1995). Kiwifruit orchards have a high water requirement, which is also influenced by differences in average daily evapotranspiration between growing areas (Holzapfel et al., 2000; Xiloyannis et al., 2023), so irrigation needs to be properly managed. In the main kiwifruit growing areas of New Zealand, effective rainfall is significantly greater than evapotranspiration and soils are free-draining with a water-holding capacity of about 400 mm, providing large amounts of water available to plants. As a result, irrigation is almost negligible in the region, with only about 30% of the kiwifruit orchards in the region irrigated (Deurer et al., 2011; Müller et al., 2015). The situation is different in other growing areas, where temperature, irradiance and leaf-to-air vapor pressure deficit are high and precipitation is low (e.g., Mediterranean regions), resulting in a large atmospheric water deficit and significant irrigation requirements (Xiloyannis et al., 2012; Dichio et al., 2013; Xiloyannis et al., 2023). In addition, other environmental factors, such as soil type, have a significant influence on crop distribution and the success of production (Salinger and Kenny, 1995).

A recent study found that climate has the most significant effect on kiwifruit growth, followed by soil and terrain conditions (Gao et al., 2022). Climate therefore plays the most important role in kiwifruit growth, with relative humidity, late frost days, days with temperature $\geq 35^{\circ}\text{C}$ and annual precipitation being the most influential conditions, indicating that water and temperature are key indices. Among the soil conditions, soil depth, pH, type and terrain aspects influence kiwifruit growth.

Kiwifruit is particularly exposed and sensitive to climatic conditions throughout the growing season and to inter-annual climate variability (Salinger and Kenny, 1995; Cradock-Henry, 2017). Regions identified as ideal for growing kiwifruit have experienced significant shifts in climatic conditions in recent years due to climate change. Combinations of temperature and precipitation, climate variability and extreme events are affecting both yield and fruit quality (Cradock-Henry, 2017), as well as the overall viability of production in major kiwifruit growing areas around the world (Tait et al., 2018; Rajan et al., 2024).

In Italy, kiwifruit is cultivated along the entire length of the peninsula, with varying environmental conditions from north to south (Testolin and Ferguson, 2009). The wide range of climatic conditions across Italy, affecting the length of the growing season, the occurrence of seasonal frosts and winter-low or summer-high temperatures in the north and south, the degree to which winter chilling requirements are met, the distribution of precipitation and the intensity of solar radiation, pose climatic challenges for kiwifruit cultivation (Testolin and Ferguson, 2009). Northern regions may suffer from intense precipitation and the occurrence of spring and autumn frosts, while southern regions are characterized by warmer winters, which limit the fulfilment of chilling requirements, and dry summers with higher evaporative demand and thus increased irrigation requirements.

Kiwifruit cultivation in Mediterranean regions, which are characterized by high risks of drought and precipitation deficits, as well as heavy precipitation associated with tropical cyclones (Medda et al., 2022), is challenged by the increased establishment of plant water stress.

Kiwifruit vines sustain high rates of transpiration and photosynthesis under non-limiting soil water conditions. However, they exhibit more conservative behavior when the soil becomes water-limited, with significant reductions in stomatal conductance and photosynthesis according to the extent of water deficit achieved (Montanaro et al., 2009; Dichio et al., 2013; Calabritto et al., 2024; Fernandes et al., 2025). Water deficit can affect normal transpiration and photosynthetic activity (Chartzoulakis et al., 1993; Gucci et al., 1996; Montanaro et al., 2007a; Mills et al., 2009; Montanaro et al., 2009) as well as fruit growth and quality (Judd et al., 1989; Miller et al., 1998) in kiwifruit.

Water excess, on the other hand, has been recognized as a key factor in triggering physiological decline in kiwifruit (Savian et al., 2020; Sofo et al., 2024). Furthermore, waterlogging is not only caused by external environmental factors (e.g., heavy rainfall, river flooding, storms), but also by overuse and/or poor management of irrigation water (i.e., inappropriate irrigation practices) (Irfan et al., 2010; Manghwar et al., 2024). In the latter case, it is well known that farmers tend to 'play it safe' and increase irrigation, especially when the associated costs are low and water resources

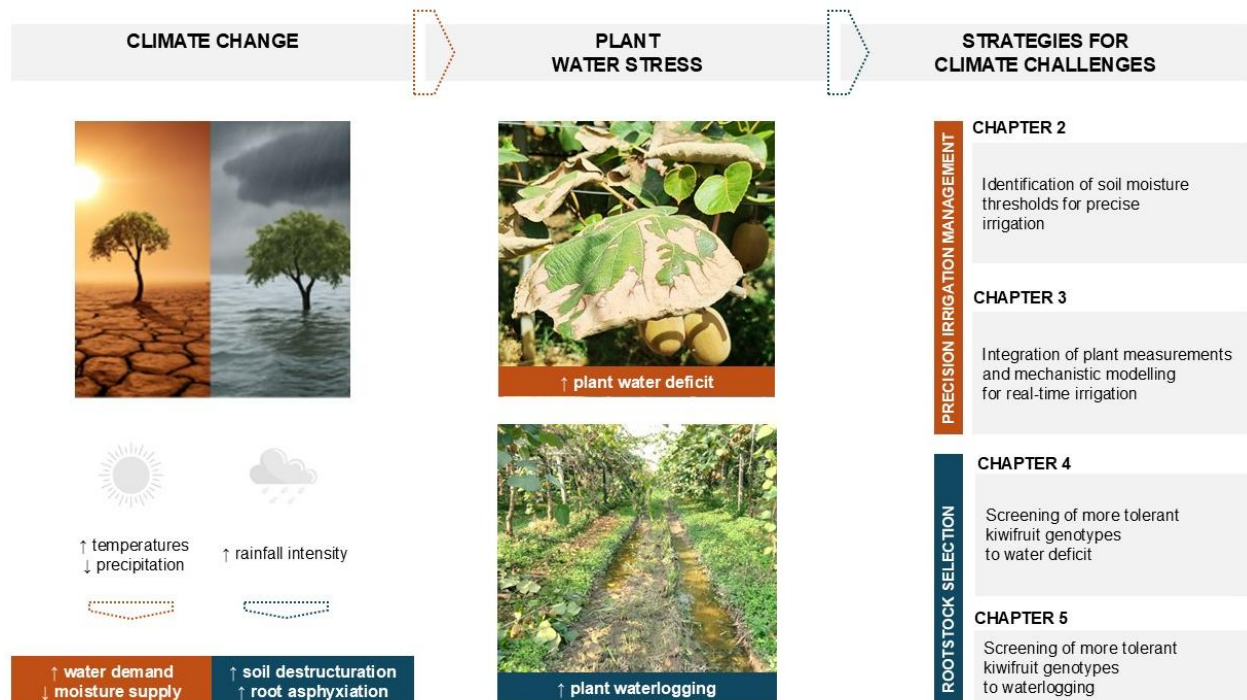
are not limited, often over-irrigating crops and promoting waterlogging conditions (Chartzoulakis and Bertaki, 2015; Kokkora et al., 2023).

In the context of a changing climate, sustainable management practices can be potential tools to mitigate and adapt to the consequences of climate change. In particular, kiwifruit orchards need to be more resilient to climate-related impacts associated with warmer temperatures, limited water resources, and increased risk of extreme events (Müller et al., 2015). In-depth studies on the vine's response to different abiotic stresses in Mediterranean environments are needed to optimize the management of kiwifruit orchards through agronomic practices that improve the resilience and productivity of kiwifruit cultivation, and mitigate the climate-related impacts. Adaptation and mitigation strategies may include:

- shade management (Montanaro et al., 2009; Boini et al., 2021) (use of shade nets to reduce sunburn and midday depression and create favorable microclimates within orchards);
- irrigation management (Torres-Ruiz et al., 2016; Ben Abdelkader et al., 2022; Dichio et al., 2024) (minimize water loss and optimize water use efficiency based on soil moisture levels, environmental conditions and plant status, split the daily irrigation volume to synchronize water supply with plant absorption and transpiration processes, develop innovative irrigation methods that allow root system development over a larger soil volume while ensuring oxygenation of the soil and roots);
- sustainable soil management (Montanaro et al., 2007b; Campi et al., 2020; Sofo et al., 2024) (cover crops, no or reduced tillage, compost addition, optimize land use, preserve biodiversity).

Optimized management strategies, combined with the breeding of more tolerant cultivars or rootstocks to abiotic stresses, have an important effect on kiwifruit production (Rajan et al., 2024), increasing the resilience of existing orchards and extending the frontiers of kiwifruit cultivation to new areas of the countries.

1.3 Thesis overview and general aim



Climate change is expected to worsen global climatic conditions, leading to higher temperatures, increased evaporative demands, water scarcity and extreme events. These conditions threaten crop production by exacerbating the emergence of new and more frequent biotic and abiotic stresses. Among these, water stress (both deficit and excess), which can be caused by climate change as well as improper irrigation management, has a serious impact on kiwifruit cultivation. In particular, kiwifruit is characterized by low tolerance to both drought and waterlogging, making it a model crop for precision irrigation.

Considering that Mediterranean regions are recognized as the main climatically challenging environments and that the global kiwifruit industry is turning towards yellow-fleshed kiwifruit cultivars, the first part of this work arises from the need to study the physiological behavior of yellow-fleshed kiwifruit vines grown in a typical Mediterranean environment.

The first objective is then to assess the physiological responses of the vines to decreasing soil water availability and to identify soil moisture thresholds for precision irrigation (Chapter 2). The definition of soil moisture thresholds allows, on the one hand, to maintain soil moisture within a range that provides the most suitable soil water environment for kiwifruit vines and, on the other hand, to avoid water losses in view of the increasing need for water saving.

Then, plant measurements monitored in continuous are integrated with a dynamic water flow model. The second objective is to characterize the hydraulic behavior of yellow-fleshed kiwifruit

vines under varying water conditions (from well-watered to more or less severe drought) in a Mediterranean environment through a modelling approach (Chapter 3). Daily derivation of plant hydraulic parameters along the water transport pathway, such as hydraulic capacitance and resistance, can help implement real-time irrigation management in Mediterranean kiwifruit orchards.

In addition to the growing need for precision irrigation management, breeding and selecting climate-resilient kiwifruit cultivars is a key strategy for addressing the impacts of adverse soil and environmental conditions on kiwifruit production. The second part of this work then focuses on comprehensively screening the differential responses of kiwifruit genotypes to water stress.

The objective is to evaluate the tolerance/susceptibility of different pot-grown kiwifruit rootstocks, as well as the responses of the yellow-fleshed kiwifruit cultivar grafted onto them, to both water deficit (Chapter 4) and water excess (Chapter 5) through an integrated physiological and phenotyping approach. In addition to the main objective of identifying more tolerant rootstocks, these studies aim to reinforce the role and support the use of phenotypic tools for a more comprehensive screening of different genotypes in kiwifruit.

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2. Spatio-temporal dynamics of root water uptake and identification of soil moisture thresholds for precision irrigation in a Mediterranean yellow-fleshed kiwifruit orchard

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Abstract

Actinidia is highly susceptible to water stress, both excess and shortage, and is therefore a model fruit crop for irrigation management, requiring precise water application. The present study was carried out in a mature kiwifruit orchard in southern Italy to investigate the physiology of a yellow-fleshed kiwifruit cultivar under non-limiting soil water conditions and in response to a progressive decrease in soil water content in a Mediterranean environment, with the aim of defining soil moisture thresholds to guide irrigation management. The progressive lowering in soil moisture was monitored using multi-profile probes, taking into account a 60 cm layer. Plant water status and physiological parameters were measured throughout the experiment and were significantly correlated with soil water status, suggesting that the level of soil water deficit affects plant physiological performance. Reference minimum values of stem water potential reached during the day under non-limiting soil water conditions ranged from -0.4 to -0.7 MPa, with a value of -0.8 MPa identifying the threshold below which stomatal conductance began to decrease significantly. Soil moisture thresholds were defined according to the spatio-temporal dynamics of available water (AW) reduction, which decreased by approx. 10% and 1% before the onset of water stress and 16% and 2% at the onset of water stress, considered in the 0-30 cm and 30-60 cm soil layers, respectively, compared to the AW content of the whole soil profile. Results confirmed that root uptake was mainly concentrated in the first 30 cm of soil depth, which should be properly managed by irrigation, as reduced soil water availability could easily lead to plant water stress. An integrated approach, combining plant measurements and soil water content monitoring, together with an assessment of root water uptake dynamics, is essential to identify soil water thresholds and develop precision irrigation, especially for high water-demanding crops and environments.

2.1 Introduction

Mediterranean environments are characterized by increasingly high temperatures, light intensities and vapor pressure deficit, combined with low precipitations, especially during the summer period. This affects the physiological behavior of plants and requires a consistent use of water for irrigation, the availability of which is severely compromised (Xiloyannis et al., 2012). The variability of climatic parameters induced by climate change affects several aspects of plants, altering phenological stages and modifying physiological processes, yield and qualitative traits (Medda et al., 2022).

In recent years, economic and market pressures have led to major investments in kiwifruit production, with more than 26,000 hectares under cultivation in Italy. Kiwifruit, which originated in China, has grown in habitats characterized by relatively high humidity, abundant rainfall and a moderate light intensity (Ferguson, 1984). Despite the climatic conditions in which kiwifruit grows, the commercial importance of this crop has encouraged its cultivation and spread in the semi-arid Mediterranean area. The most commercially important and widespread kiwifruit belongs to the genus *Actinidia*, in particular the green-fleshed variety (*A. chinensis* var. *deliciosa*), which prefers cold climates and higher altitudes (from western China), is gradually being replaced by the yellow-fleshed variety (*A. chinensis* var. *chinensis*), which is better adapted to warm climates (from eastern or southern China) (Ferguson, 2013; Bardi, 2020).

Kiwifruit has evolved large leaves (wide transpiring surface) and conducting xylem vessels as an adaptation to environments with high soil water availability (Dichio et al., 2013), showing a very low resistance to water movement within the vine and a high transpiration rate (McAneney and Judd, 1983; Chartzoulakis et al., 1993). Numerous tiny fibrous (feeder) roots developing from thicker structural and secondary roots constitute the kiwifruit root system, with high root density and intense development in the upper soil layers, which decrease with increasing soil depth (Gandar and Hughes, 1988).

Due to its anatomical features, kiwifruit is widely recognized as a water-consuming plant with high water requirements (Chartzoulakis et al., 1993; Torres-Ruiz et al., 2016), but at the same time extremely sensitive to water stress, either as water excess (Savian et al., 2020; Sofo et al., 2024) or shortage (Montanaro et al., 2007; Dichio et al., 2013). Water requirement is influenced by environmental and soil characteristics as well as by plant morphology and physiology (Holzapfel et al., 2000). Optimal water application levels in kiwifruit orchards allow for normal transpiration, photosynthetic activity and overall physiological functioning of the plant. Due to the high sensitivity of kiwifruit to different levels of water application, special attention should be paid to irrigation management to ensure water distribution in the right amount, at the right time and in the

right place in order to improve the physiological performance of kiwifruit vines and to develop new and more efficient precision irrigation strategies for this crop.

On the one hand, optimized irrigation management and proper scheduling of the amount and timing of water supply can be achieved by evaluating plant water status (Virnodkar et al., 2020). Plant water status represents a sensitive indicator of water stress, measuring the plant response to the combined effects of soil moisture availability, environmental evaporative demand, hydraulic resistances and root uptake capacity (Ihuoma and Madramootoo, 2017). Predawn and midday water potentials have been the most commonly used indicators of plant water status and stress for irrigation scheduling in fruit orchards (Naor and Cohen, 2003).

On the other hand, soil water content monitoring has recently become a key factor in irrigation optimization, as it provides important information for better estimation of crop water demands (Karandish and Šimůnek, 2016), and sensor-based soil moisture measurements seem to effectively improve irrigation water application (El-Naggar et al., 2020). Changes in soil water content in the root zone, which reflect the combined history of root water uptake and recent wetting by irrigation, can be effectively monitored using soil moisture probes (Clothier and Green, 1994). Plants take up soil water in the root zone, gradually depleting the available water to meet the evapotranspiration needs (Ihuoma and Madramootoo, 2017), which are considered to be unreduced as long as the readily available water (RAW) is used by plants (Ferreira et al., 2017). Therefore, efficient irrigation management also depends on knowing the spatial and temporal distribution of root water uptake, as well as predicting changes in soil water status in the root zone after irrigation (Koumanov et al., 2006), which can be easily detected by soil moisture probes. Consideration of the soil volume explored by the root system is a key issue in defining the most effective irrigation strategy (McAneney and Judd, 1983), as the distribution of fine roots affects the potential water uptake activity, so it might be expected that the pattern of uptake would be similar to the distribution of fine roots under non-limiting water conditions (Green and Clothier, 1995). The hyperactivity of the near-surface roots is revealed by the rapidly changing trends in soil water content monitored in the first layers, approximately 0-20 cm (Green et al., 2006). An initial good correspondence between water uptake and root density was found under well-watered conditions in the rootzone, while as water stress developed, the deeper layers, where water was more freely available, began to contribute an increasing fraction of total water uptake, despite their lower root density (Green and Clothier, 1995). While there are some investigations on kiwifruit root morphology and distribution (Greaves, 1985; Gandar and Hughes, 1988; d'Andria et al., 1991), more information is needed to improve knowledge of the spatial and temporal distribution of root water uptake, especially under different localized irrigation application strategies.

Soil water content thresholds, defined as the lower or upper limits of soil water content for irrigation, are important to provide the most suitable soil water environment for optimal kiwi performance (He et al., 2023) and should take into account water uptake dynamics.

Soil water observations combined with plant measurements and responses are essential for implementing suitable and more accurate irrigation scheduling methods (Intrigliolo and Castel, 2004), requiring early detection of water stress (Green et al., 2015).

Conflicting opinions have been reported on stomata control and the ability of kiwifruit to reduce water loss under water restriction. Stomatal regulation, which is involved in the control of water loss and plant water potential during water stress, differs among species but also between cultivars due to adaptive responses that have evolved to limit severe drops in potentials (Álvarez-Maldini et al., 2022), leading to the distinction between isohydric and anisohydric behavior. Gucci et al. (1996) found that stomata were highly sensitive to changes in soil water content, effectively regulating transpiration processes in Hayward kiwifruit. In contrast, other research has shown that leaves were unable to regulate stomatal conductance with increasing soil water deficit, continuing to lose water (Judd et al., 1989). It is still unclear whether and how drought affects the physiological responses and stomatal behavior of kiwifruit at the field scale. As soil water potential decreases, leaf stomata influence gas exchange dynamics and the feedback mechanism between plant water potential and stomatal conductance is crop-specific and varies among species (Jamshidi et al., 2020). While most studies on water relations and plant physiological processes have been carried out on the well-known green cultivar 'Hayward', less is known about the yellow cultivars and associated crop management protocols (Mills et al., 2009). Mills et al. (2009) reported better stomatal control under water stress in yellow kiwifruit compared to the green cultivar, while Black et al. (2012) found reductions in Ψ_{leaf} without significant reductions in stomatal conductance, noting that the observed reductions in Ψ_{leaf} were insufficient to induce a stomatal response. Previous studies have found that the anisohydric or isohydric behavior of cultivars also depends on soil hydraulic conductivity and water content (Tramontini et al., 2014), suggesting a great influence of the environment and a plant-environment interaction rather than an intrinsic property of the plant behind the discriminated behavior (Hochberg et al., 2018).

The combination of soil water status, which shows a direct relationship with the amount of irrigation water applied (Holzapfel et al., 2000), with plant physiological responses could effectively help to modulate and manage the water content in the soil profile to ensure improved plant functioning. Therefore, the main objectives of this study were: (1) to further characterize the physiology of yellow-fleshed kiwifruit under non-limiting soil water availability in a typical Mediterranean environment; (2) to evaluate the effects of progressive soil water depletion on the

behavior of yellow-fleshed kiwifruit in order to identify RAW thresholds for precision irrigation that ensure optimal plant physiological performance, and levels below which plant water status and physiological parameters are significantly affected; (3) to investigate the spatial pattern of root water uptake, considering the dynamics of available water (AW) depletion, to develop and match precision irrigation strategies to the pattern of plant water extraction from the soil profile.

2.2 Material and methods

2.2.1 Experimental site and plant material

The experimental trial was carried out during the 2022 growing season over a 4-week period from 23 August to 19 September (DOE=days of the experiment) in a 9-year-old mature commercial kiwifruit (*Actinidia chinensis* var. *chinensis*, cv. Zesy002) orchard in Metaponto, southern Italy (latitude 40°24'N, longitude 16°46'E). The yellow-fleshed cultivar was grafted onto D1 rootstock (*A. chinensis* var. *deliciosa*), cultivated at 5 m x 2 m spacing and trained as a pergola system. The soil profile consisted of a uniform sandy clay loam texture (58% sand, 14% silt and 28% clay). Irrigation was provided by micro-sprinklers, using a single emitter per vine spaced 1 m from the trunk, at a flow rate of 40 L h⁻¹ and with a wetted radius of 0.9 m. Soil volume wetted by irrigation, expressed as total volume per hectare (m³ ha⁻¹), was assumed as a cylinder and calculated considering the sprinkler wetting radius, the depth of the wetting pattern (0.6 m) and the number of emitters per hectare.

All vines were grown under optimal water availability conditions during the season until the beginning of the experimental trial. Daily vine water requirement was estimated through the compilation of the water balance, taking into account environmental data and crop coefficient (K_c) reported in the FAO Irrigation and Drainage paper Nr. 66 (Steduto et al., 2012). The scheduled irrigation volume was adjusted throughout the season through the soil water balance, operating feedback adjustment mechanisms based on the monitoring of the water content in the soil volume wetted by irrigation by soil moisture probes, in order to maintain the soil moisture level close to or slightly below the field capacity (FC) (Vera et al., 2019; Mininni et al., 2022). Thus, irrigation drainage in this study was considered negligible. The experimental irrigation-managed plot, consisting of 63 vines, was equipped with digital flow meters and an automatic irrigation controller for remote control of water supply ("Irrifarm" system, Pan. Agri s.r.l., Basilicata, South Italy). Daily irrigation volumes ranged from 30 to 60 m³ ha⁻¹ during the season.

From 23 August on, 12 vines within the experimental plot and distributed along a row, underwent restricted irrigation (WS) with irrigation volumes reduced by 30% and ceased, adjusted taking into account rainfall contribution, to allow the soil to achieve a progressive available water content

(AW) depletion. The soil about the control vines (C) received optimal irrigation, ensuring a soil water content level close to or slightly below the FC, so that it remained well-watered throughout the experiment. Half of the water-stressed vines were re-irrigated after 15 days (R_I) following the irrigation strategy kept for the control vines, restoring the available water content in the soil profile, while the remaining half of the vines continued to be subjected to restricted irrigation for other 5 days (WS), subsequently entering the recovery phase (R_{II}).

2.2.2 Meteorological data and soil water status monitoring

A meteorological station was installed nearby the experimental orchard block and mounted above the kiwifruit canopy, outside the hail net protection. The sensors recorded hourly averages of wind speed, air temperature, relative humidity, evapotranspiration and daily rainfall. Hourly air vapor pressure deficit (VPD) was computed using air temperature and relative humidity.

Frequency Domain Reflectometry (FDR) 90 cm multi-profile soil moisture probes (Drill & Drop, Sentek Sensor Technologies Stepney, Australia) were used to continuously monitor the volumetric soil water content in 10 cm increments throughout the soil vertical profile, allowing the remote detection of instantaneous oscillations and trends in each soil layer. A soil layer of 0-60 cm was considered useful for irrigation scheduling in kiwifruit (Xiloyannis et al., 2023). Sensors were installed on the row at a distance of 0.5 m from the vine stem and approximately in the center of the volume of wet soil. Measured values of soil moisture were stored in 15-min intervals using a data logger (Model CR1000, Campbell Scientific, USA), hourly means were considered for analyses.

2.2.3 AW depletion and spatial pattern of root-water uptake

The soil physico-hydrological properties were evaluated through the soil-water retention curve obtained with the Richards' pressure plates apparatus (Richards, 1948) to define the soil-water relation and, in particular, the quantity of water held at different values of soil matric potential, and therefore the FC, the wilting point (WP) and the AW in the whole profile (0-60 cm) (Table 1). The available water (AW, $m^3 ha^{-1}$) that can be contained in each layer, and thus in the whole 0-60 cm soil profile considered in the present study, was calculated taking into account soil water retention characteristics derived from the soil-water retention curve (FC and WP values, Table 1) and soil volume of each 10 cm layer wetted by irrigation calculated on a per hectare basis, which is influenced by the irrigation system and the wetted radius. The volumetric soil water content values recorded by the soil moisture probes in 10 cm increments were used to estimate the actual available water (AW_{act} , $m^3 ha^{-1}$) contained in each soil layer and in the whole 0-60 cm profile on

a per hectare basis. The depletion of AW (AW_{depletion}, %) was calculated using the following equation:

$$AW \text{ depletion (\%)} = \frac{AW - AW_{act}}{AW} * 100$$

where AW is the potential available water contained in each soil layer (10 cm increments) or in the whole profile (0-60 cm) and AW_{act} is the actual value of the available water calculated for each day of the experiment.

Table 1 Soil physico-hydrological characteristics and available water contents in the soil profile (0-60 cm) and in different soil layers (0-20, 20-40, 40-60 cm).

Soil properties	Value
Soil volume wetted by irrigation (m ³ ha ⁻¹)	1,526
Bulk density (kg dm ⁻³)	1.39
Field Capacity (cm ³ cm ⁻³)	0.39
Wilting Point (cm ³ cm ⁻³)	0.18
Available Water (m ³ ha ⁻¹) (0-60cm)	320.30
Available Water (m ³ ha ⁻¹) (0-20 cm)	106.77
Available Water (m ³ ha ⁻¹) (20-40 cm)	106.77
Available Water (m ³ ha ⁻¹) (40-60 cm)	106.77

FDR technologies, which allowed for routine measurements and detection of temporal changes in the soil water content over time, were used to deduce the spatial pattern of water uptake by the roots in the soil profile. In Mediterranean environments, the volume of soil not wetted by irrigation tends to dry out during the warmer months, so it is not affected by root water uptake (Green and Clothier, 1995). Drainage of water in the deeper layer was nullified by the precise irrigation management, which was based on the continuous monitoring of soil water content and the distribution of daily irrigation volumes in two/three events per day, thus it was considered negligible in the present study. Soil evaporation plus understorey transpiration were minimized due to localized micro-irrigation, high vine cover and absence of cover crops, making irrigation requirements almost dominated by vine transpiration (Goodwin et al., 2004; Goodwin et al., 2015). Therefore, they were assumed to be not relevant for irrigation management purposes in the present study.

2.2.4 Plant water status and leaf gas exchange

Diurnal courses of physiological stress indicators were measured to investigate plant-water relations by analyzing their trends influenced by changing soil water status conditions, both for stressed and well-watered vines, and considering the peculiar physiological and anatomical traits of kiwifruit vines.

Physiological measurements were performed during the increasing soil water deficit conditions (WS vines) and the subsequent recovery phases (R_I and R_{II} vines) compared to C vines.

Plant water status and stress level were monitored through the stem water potential (Ψ_{stem} , MPa) on six fully developed, expanded and not exposed to sunlight leaves chosen from the middle part of the canopy of three plants per treatment. The selected leaves were wrapped in a tin foil for about half an hour and then excised for immediate measurement with a Scholander pressure chamber (Model 1000, PMS Instrument Company, Corvallis, OR, USA) pressurized with nitrogen according to the procedure recommended by Begg and Turner (1970). The Ψ_{stem} daily trends were monitored on seven days of the experiment at two-hourly intervals throughout the day, from 5:30 (pre-dawn values) to 16:30 (afternoon values) hours (European daylight savings time, DST), in order to understand the synchronization of Ψ_{stem} diurnal pattern with soil volumetric water content and VPD environmental conditions. Stomatal conductance (g_{sw} , $\text{mol m}^{-2} \text{s}^{-1}$), transpiration (E , $\text{mmol m}^{-2} \text{s}^{-1}$) and leaf temperatures (T_{leaf} , °C) were similarly monitored during the same days with a portable handheld LI-600 porometer system integrated with a fluorometer (LI-COR Biosciences, Lincoln, USA). Values obtained were averages of nine measurements taken on mature and sun-exposed leaves, near the top of the canopy, with no visual symptoms of biotic or abiotic stress, from three vines per treatment. During the other days (five) of trial observation, measurements of Ψ_{stem} , g_{sw} , E and T_{leaf} were conducted at midday (between 12:30 and 14:30 hours) on sunny days. Leaf to air temperature differences (ΔT (°C) = $T_{\text{leaf}} - T_{\text{air}}$) were calculated. Measurements of Ψ_{stem} , g_{sw} and E were carried out at 1, 2, 5, 7, 12 and 2, 7 days after re-watering for the first and second recovery phases (R_I and R_{II}) compared to C. Measurements of photosynthesis (A , $\mu\text{mol m}^{-2} \text{s}^{-1}$) were carried out on six fully expanded, developed and sun-exposed leaves from three vines during the drought (diurnal trends for four days) and re-watering (midday values for two days) cycles with an open gas exchange system unit (ADC LCA-4, Analytical Development Co., Hoddesdon, UK).

2.2.5 Statistical analysis

All statistical analyses were performed using RStudio statistical software (4.2.2 version, Posit Software, PBC, Boston, MA) and results were plotted with SigmaPlot 15.0 (Systat Software Inc.,

San Jose, CA, USA). Data were reported as mean value and standard error of the mean ($\pm$ SE). The data were subjected to an analysis of variance (one-way ANOVA) to examine the differences between irrigation treatments at each sampling time (p value <0.05 was considered significant) after testing for normality distribution of residuals (Shapiro-Wilk test) and homogeneity of variances (Levene's test). Dunnett's test was used for mean comparison of WS and R_I against the control. To investigate the relationships between physiological indicators (Ψ_{stem} , g_{sw} , A) and soil water status (SWC), polynomial regression models were fitted to averaged data from each irrigation treatment.

2.3 Results

2.3.1 Environmental conditions and progressive soil water depletion

The experimental trial coincided with a period characterized by a quite high evaporative demand. The weather was generally sunny and dry with the exception of a few days when rainfall occurred. 19 days out of the 28 days had maximum air temperatures that exceeded 30 °C (Figure 1), while the minimum air temperatures varied between 9 and 24 °C during the experimental trial. VPD was quite variable throughout the experimental days, reaching maximum hourly values equal to 3.65 kPa at DOE 3. Daily ET_0 ranged between 3.5 and 6.0 mm. Daily net solar radiation reached a maximum value of 24.2 MJ m⁻² (DOE 3) and ranged between 16.2 and 24.2 MJ m⁻² during the experimental trial. Irradiance, air temperature and humidity were typical of Mediterranean late summer conditions.

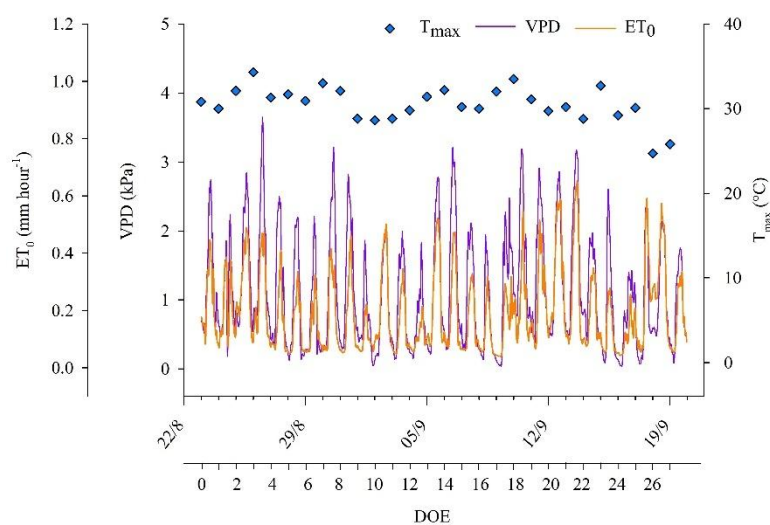


Figure 1 Hourly reference evapotranspiration (ET_0), hourly vapor pressure deficit (VPD) and daily maximum air temperatures (T_{max}) during the days of the experiment (DOE) carried out in the 2022 season.

Soil water content gradually decreased in the field area subjected to water restriction and trends of AW depletion in different soil layers and in the whole soil profile are shown in Figure 2A. In particular, AW was reduced by approx. 61%, 19% and 14% in the 0-20, 20-40 and 40-60cm soil layers, respectively, at the end of the first phase of restricted irrigation (DOE 15) before the stress was relieved by rewatering vines entering the R_I recovery phase. An AW depletion of approx. 69%, 21% and 19% computed in the 0-20, 20-40 and 40-60 cm soil layers, respectively, was achieved at the maximum level of stress (DOE 20) before the stress was relieved by re-watering vines entering the R_{II} recovery phase (Figure 2A). In C vines soil water content was on average maintained close to or slightly below the FC. As water stress increased and in response to progressive soil water depletion, Ψ_{stem} of WS vines became more negative, reaching predawn values (Ψ_{predawn}) of -0.5 and -1.0 MPa, and midday values (Ψ_{midday}) of -1.4 and -1.8 MPa, respectively at the end of the first and the second phase of restricted irrigation, when about 31% and 36.5% of AW in the whole soil profile (0-60 cm) was depleted (Figures 2A, B). C vines maintained Ψ_{stem} values indicative of an optimal plant water status, ranging from -0.1/-0.2 MPa at pre-dawn to -0.4/-0.7 MPa at midday throughout the days of the experiment of restricted irrigation (Figure 2B).

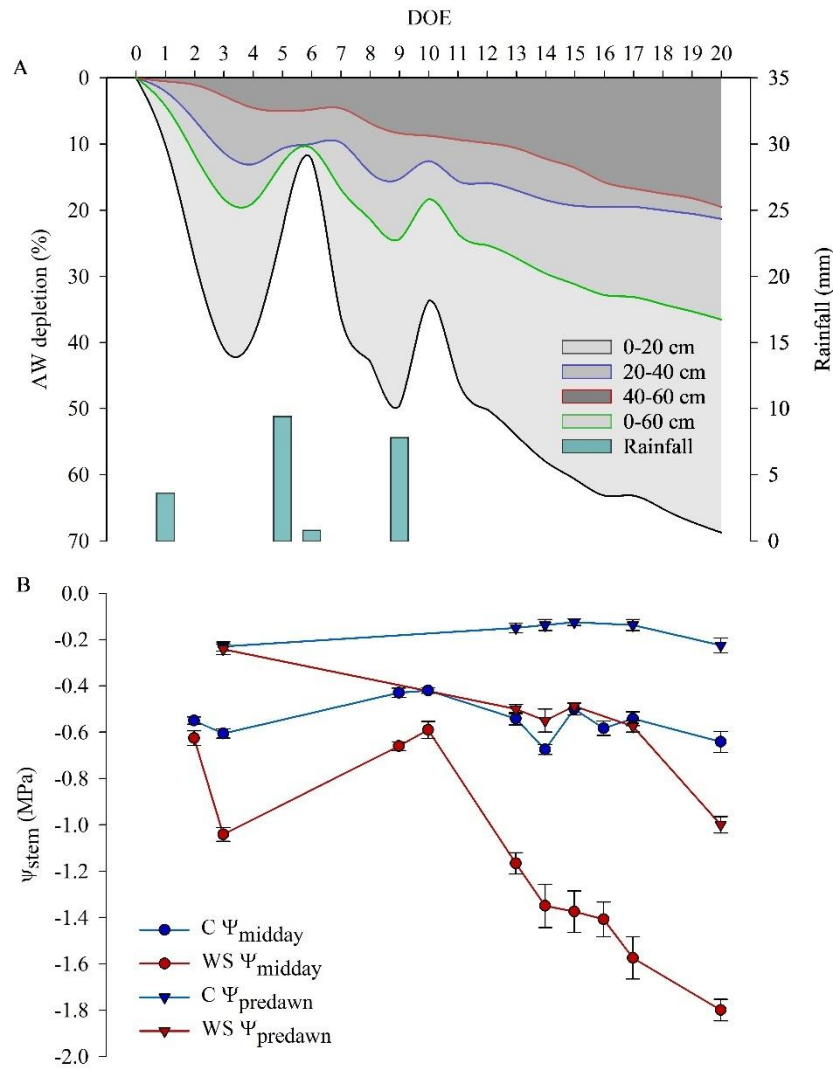


Figure 2 Daily rainfall and available water (AW) depletion averaged in the layers 0-20, 20-40, 40-60 cm and in the whole soil profile 0-60 cm (A), daily midday and pre-dawn stem water potential measured in control (C) and water stressed (WS) vines (B) during the days of the experiment (DOE) of restricted irrigation.

2.3.2 Plant water status and leaf gas exchange

Under non-limiting soil water conditions, Ψ_{stem} and ΔT observations correlated with midday VPD more tightly than E, whereas the relation between gsw and VPD was not significant (Figure 3). Relationships between physiological indicators and air VPD indicated that, under non-limiting soil water conditions, vines did not implement any stomatal regulation with the daily increase in environmental evaporative demand (i.e. VPD) preferring to transpire, losing water derived from root uptake, and slightly lowered the plant water potentials.

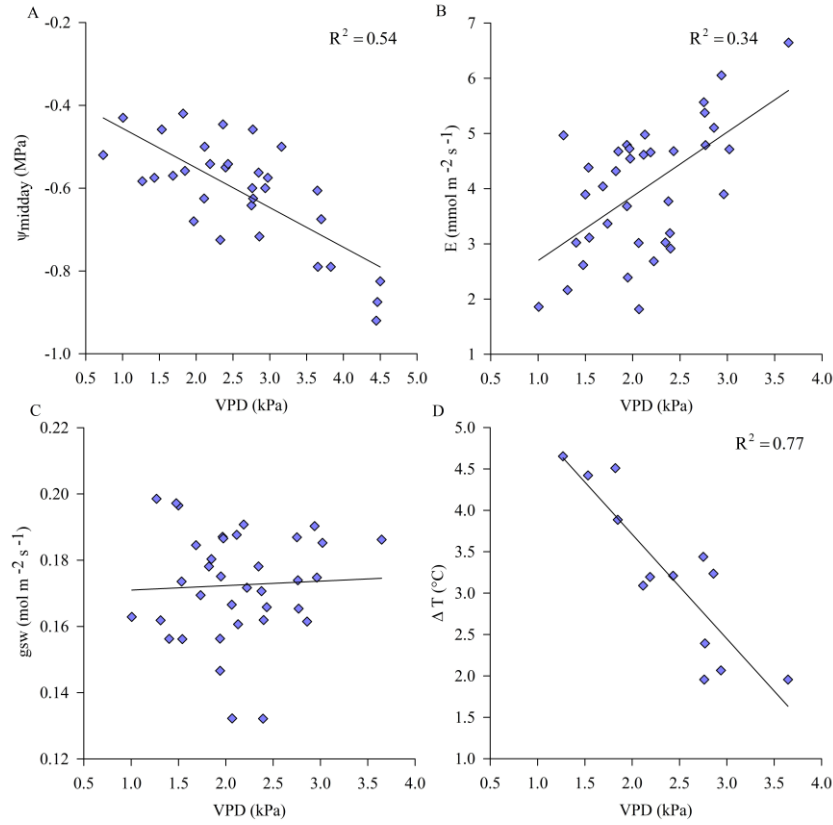


Figure 3 Variations of midday stem water potential (Ψ_{midday}) (A), transpiration (E) (B), stomatal conductance (g_{sw}) (C) and midday leaf to air temperature differences (ΔT) (D) to air vapor pressure deficit (VPD) under non-limiting soil water availability (well-irrigated kiwifruit vines). Symbols are means of measured data (Ψ_{stem} , $n=6$; E , g_{sw} and T_{leaf} , $n=9$).

Ψ_{stem} of C vines showed daily trends reflecting daily VPD conditions, reaching minimum values during the hottest hours (e.g., around midday), but remaining in an optimal range when soil water availability was not limiting (Figure 4), allowing the plant's physiological activities not to be compromised. Vines under limited soil water availability conditions showed a fast drop in Ψ_{stem} at midday and a gradual incapacity to recover plant water status in the afternoon hours, as highlighted on DOE 15 (Figure 4A), affecting the ability to recover during the nighttime hours, as shown by the progressive lowering of the Ψ_{predawn} values (Figures 2B, 4). Stomatal conductance remained relatively constant between 0.16 and 0.20 $\text{mol m}^{-2} \text{s}^{-1}$, throughout the day and between different days in C vines (Figure 4), supporting the results from Figure 3. Stomatal conductance of WS vines was higher early in the morning, especially during the first phase of restricted irrigation (Figures 4A, B) when Ψ_{stem} was not yet the seriously limiting factor, then gradually decreased and remained low throughout the day, reduced by approx. 55-75% compared with C vines during the days of experiment of significant stress (Figures 4C, D), reaching values relatively constant

between 0.05 and 0.08 mol m⁻² s⁻¹, probably due to the implementation of an adaptive strategy involving stomatal control as water is depleted in the root zone. However, stomatal closure, occurring in the WS vines after the early morning hours, did not prevent Ψ_{stem} from a further drop in the afternoon hours (Figures 4A, B). Conversely to gsw, E measured in kiwifruit vines under non limiting soil water conditions was affected by VPD, increasing when environmental conditions became more water-demanding (Figure 3B). Transpiration rate in C vines was low in the early morning due to lower light intensity and air VPD. With increasing light intensity and temperature, E rose reaching higher values at midday and then gradually decreased (Figure 4). In WS vines, E generally remained at very low values throughout the day, reflecting the same daily trend as in C vines. In particular, E of WS vines measured at early morning were similar to those of C vines in the first phase of restricted irrigation, also aided by low environmental demand, while they significantly differed during hours of higher evaporative demand (Figures 4A, B). Instead, E of WS vines differed since early morning from that of C vines in the second phase of restricted irrigation (Figures 4C, D), achieving a reduction of approx. 50-65% compared with control, almost constant over the days.

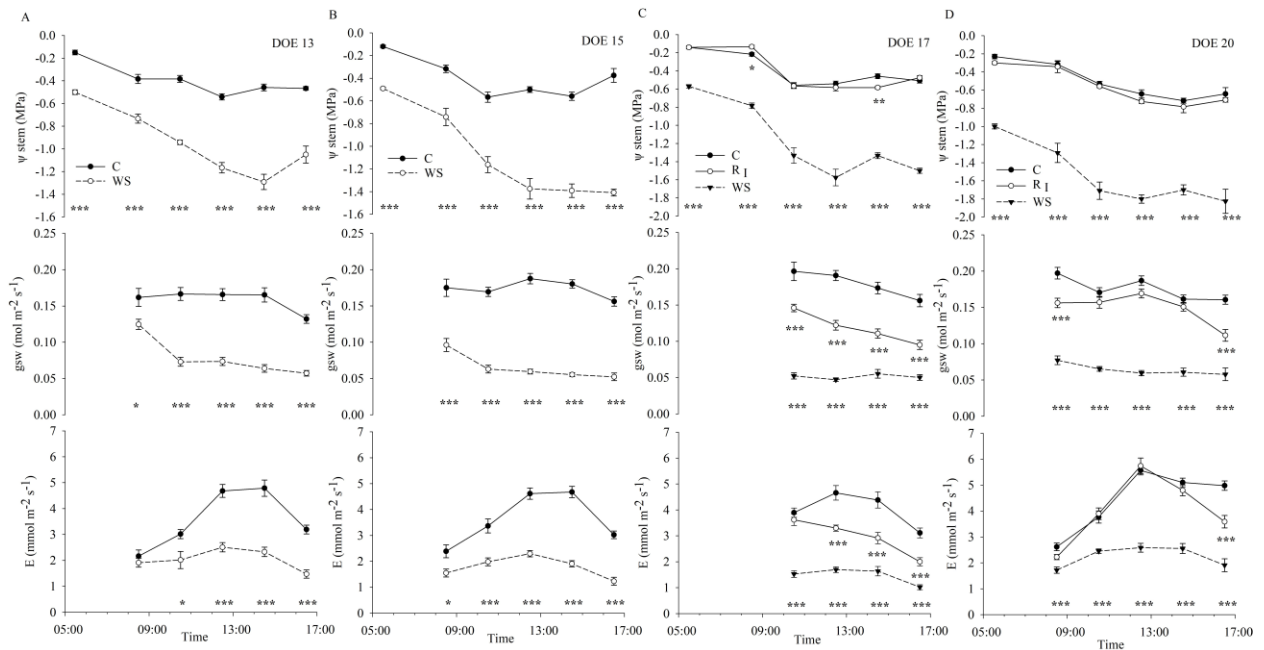


Figure 4 Air vapor pressure deficit (VPD) values and daily trends of stem water potential (Ψ_{stem}), stomatal conductance (gsw) and transpiration (E) measured in the first phase of restricted irrigation (DOE 13 (A) and DOE 15 (B); C= Control and WS= Water-Stressed) and in the second phase of restricted irrigation and first recovery (DOE 17 (C) and DOE 20 (D); C= Control, R_I= Recovery and WS= Water-Stressed). Each point is the mean of measured data $\pm$ SE (Ψ_{stem} , n=6; gsw and E, n=9). Asterisks denote significant differences in Ψ_{stem} , gsw and E, respectively, between R_I and WS vines compared with C according to a One-Way ANOVA test ($p < 0.05$).

Leaf temperature of sun exposed leaves of kiwifruit vines under non-limiting soil water conditions, monitored during the midday hours (12:30-14:30 hours), was higher than air temperature in the range of approx. 2-4.5 °C, depending on daily VPD conditions. In particular, leaf to air temperature differences (ΔT) decreased with increasing VPD and E, due to the potential cooling effect of transpiration (Figure 3D). Otherwise, in WS vines T_{leaf} were nearly 4 °C higher than in C vines on some days of experiment (data not shown) and ΔT , on average equal to approx. 6 °C, increased due to the reduced transpiration rate and consequent loss of thermoregulatory capacity under water stress conditions (Figure 5B). Reference values of Ψ_{midday} , allowing maximum stomatal activity and opening, ranged from -0.4 down to -0.7 MPa, under non-limiting soil water conditions (Figure 5A). Results showed that under a threshold value of Ψ_{midday} , generally corresponding to the minimum reached during the day, of approx. -0.8 MPa, gsw began to decrease significantly while ΔT increased (Figure 5), indicating a change in plant behavior when subjected to water stress conditions.

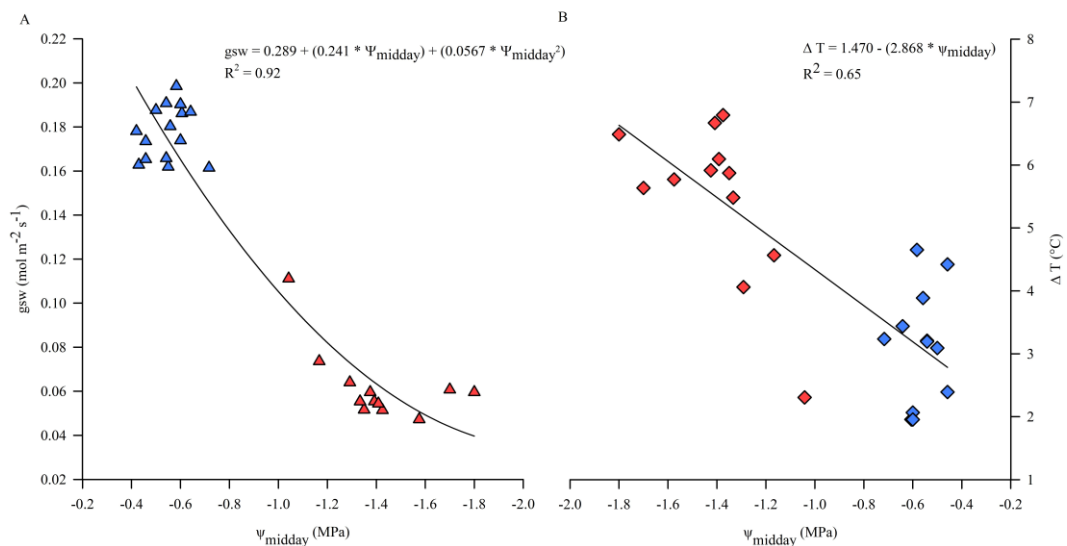


Figure 5 Correlation between midday stem water potential (Ψ_{midday}) and stomatal conductance (g_{sw}) (A) and leaf to air temperature differences (ΔT) (B) during the experimental trial. Symbols are means of measured data, blue for optimal irrigation level and soil water availability; red for restricted irrigation and progressive soil water content lowering. Line corresponds to modeled data.

Vines were then subjected to a recovery phase (R_I and R_{II}) from a different level of water stress, indicated by Ψ_{midday} and Ψ_{predawn} values of approx. -1.4 and -0.5 MPa reached at the end of the first phase of restricted irrigation, and -1.8 and -1.0 MPa reached at the end of the second phase of restricted irrigation, respectively. Recovery dynamics of physiological indicators are shown in Table 2. Upon re-watering, recovery of Ψ_{stem} was rapid both in the first and in the second recovery

(R_I and R_{II}), restoring values equal to those of C vines in the immediate 24 hours, regardless of the water stress level achieved. However, gsw of WS vines showed a delay in recovery, reaching 86% and 51% of the value of C vines one week after re-watering from the first and the second phase of restricted irrigation, respectively, showing a different recovery trend depending on the stress level achieved. Similarly, E of WS vines recovered slowly, but not as slowly as gsw, showing no differences with C vines and reaching 76% of the value of C vines 5 days and one week after re-watering from the first and the second phase of restricted irrigation, respectively (Table 2).

Table 2 Midday stem water potential (Ψ_{midday}), stomatal conductance (gsw) and transpiration (E) measured in the first (R_I) and in the second recovery (R_{II}) at several days after re-watering from a condition of pre-dawn stem water potential (Ψ_{predawn}) values of approx. -0.5 and -1.0 MPa, respectively. Asterisks denote significant differences in Ψ_{midday} , gsw and E, respectively, between R_I and R_{II} vines compared with control vines (C) according to a One-Way ANOVA test ($p < 0.05$).

Recovery days	Treatment	gsw (mol m ⁻² s ⁻¹)	E (mmol m ⁻² s ⁻¹)	Ψ_{midday} (MPa)
	C	0.199 ± 0.01	4.97 ± 0.20	-0.58 ± 0.03
1	R _I	0.107 ± 0.01	2.69 ± 0.22	-0.56 ± 0.02
	Significance	***	***	ns
	C	0.191 ± 0.01	4.66 ± 0.28	-0.54 ± 0.03
2	R _I	0.122 ± 0.01	3.30 ± 0.12	-0.58 ± 0.04
	Significance	***	***	ns
	C	0.187 ± 0.01	5.57 ± 0.17	-0.64 ± 0.05
5	R _I	0.169 ± 0.01	5.74 ± 0.30	-0.73 ± 0.02
	Significance	ns	ns	ns
	C	0.187 ± 0.01	4.72 ± 0.17	-0.68 ± 0.02
7	R _I	0.161 ± 0.01	4.26 ± 0.23	-0.66 ± 0.04
2	R _{II}	0.065	1.99 ± 0.16	-0.75 ± 0.05
	Significance C vs. R _I	**	ns	ns
	Significance C vs. R _{II}	***	***	ns
	C	0.185 ± 0.01	4.04 ± 0.22	-0.57 ± 0.03
12	R _I	0.150 ± 0.01	3.33 ± 0.20	-0.64 ± 0.02
7	R _{II}	0.095 ± 0.01	2.52 ± 0.18	-0.80 ± 0.05
	Significance C vs. R _I	**	*	ns
	Significance C vs. R _{II}	***	***	**

The response of physiological indicators to SWC during the days of experiment is shown in Figure 6. In particular, Ψ_{midday} , g_{sw} and photosynthesis (A) showed a positive correlation with SWC recorded by soil moisture probes in the 0-40 cm soil layer, indicating that a progressive decrease in SWC negatively affected plant behavior (Figure 6). Relationships were carried out considering the 0-40 cm soil layer that was mainly affected by root water uptake dynamics as shown in Figures 2A, 8. SWC was maintained approximately in the range between 40% and 50% for C vines in the 0-40 cm soil layer. Stomata were wide open, allowing maximum transpiration rates when SWC was maintained at optimal levels (Figure 6B). Ψ_{midday} , g_{sw} and A declined by 50% once SWC dropped to about 27%, 26% and 24%, respectively. At much lower SWC, the stomata were almost completely closed, which severely affected vine transpiration. Determination coefficients (R^2) of the relationships between physiological indicators and SWC were higher for g_{sw} , followed by Ψ_{midday} and A, equal to 0.84, 0.73 and 0.64, respectively. The intersection of the mean value of SWC calculated for the control vines with the function trajectory resulted in a reference value of -0.67 MPa, $0.17 \text{ mol m}^{-2} \text{ s}^{-1}$ and $16.0 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ for Ψ_{midday} , g_{sw} and A, respectively (Figure 6).

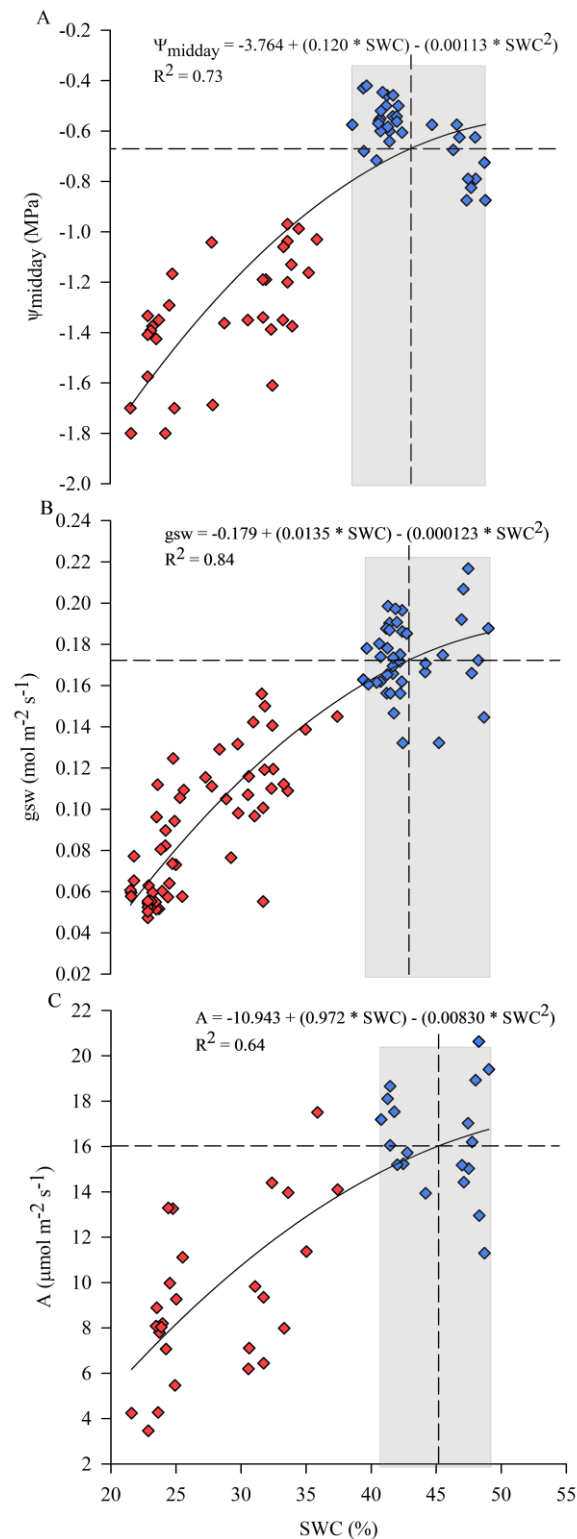


Figure 6 Responses of midday stem water potential (Ψ_{midday}) (A), stomatal conductance (g_{sw}) (B) and photosynthesis (A) (C) to soil water content (SWC) considered in the 0-40 cm soil layer during the experimental trial. Symbols are means of measured data, blue for optimal irrigation level and soil water availability, red for restricted irrigation and progressive soil water content lowering. Lines correspond to modeled data. (Some additional measurements taken externally to the experimental period were included in the analysis).

The analysis of plant measurements, carried out in the WS vines during the days of experiment of restricted irrigation, in relation to the progressive AW depletion reached in different soil layers (0-20, 20-40 and 40-60 cm) is shown in Figure 7. Ψ_{midday} and gsw showed to be greatly affected by the dynamic of AW depletion according to soil depth, in particular, changes in plant water status and stomatal behavior are associated with higher reductions of the available water contained in the 0-20 cm soil layer, followed by the 20-40 cm and 40-60 cm soil layers (Figures 7A, B).

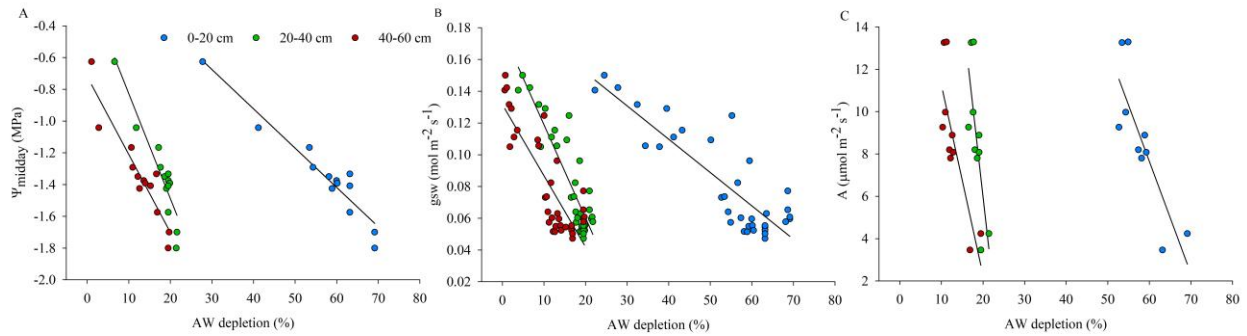


Figure 7 Distribution of midday stem water potential (Ψ_{midday}) (A), stomatal conductance (gsw) (B) and photosynthesis (A) (C) of water-stressed (WS) vines in relation to available water (AW) depletion (%) considered in the 0-20, 20-40 and 40-60 cm soil layers during the experimental trial. Lines are only illustrative.

2.3.3 Water uptake by roots

Daily SWC and AW depletion trends in C and WS vines, respectively, are shown in Figure 8. The changing pattern of SWC around the C vines reflected the combined effects of multiple daily irrigation interventions and the root water uptake strategy used by the vine. Greater variations in SWC were observed at 15, 25 and 35 cm soil depths (Figure 8A), while at deeper depths an almost flat trend in SWC was observed (data not shown). Three daily irrigation interventions were carried out to allow the vine to meet more efficiently the transpiration demand, which was higher during the midday hours, as shown by the increased root water uptake in the first 15 cm of soil on both days, but particularly on DOE 13, probably due to the increased environmental evaporative demand (Figures 8A, 1).

The progressive decrease in SWC around the WS vines reflected a differential reduction in AW according to different soil depths and time of the day. Water uptakes were mostly concentrated in the central hours of the day (i.e., from 6:00 to 18:00), as shown by the decrease in AW contents when the vine accessed water to meet transpiration demands (Figure 8B). Preferential root water uptakes occurred in the superficial soil layers, as significant reductions of approx. 3.6%, 2.4% and 1.5% were achieved at 15, 25 and 35 cm soil depths during DOE 13, indicating a decrease in AW

depletion with increasing soil depth (Figure 8B). Water uptake in the deeper layers increased gradually and slowly as the AW in the superficial soil layers was depleted.

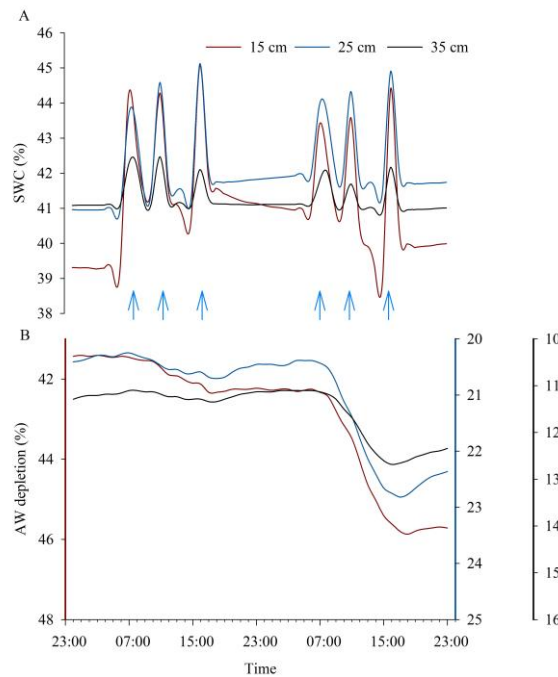


Figure 8 Daily courses of volumetric soil water content (SWC) in control vines (A) and of available water (AW) depletion in water-stressed vines (B) at 15, 25 and 35 cm soil depths on two consecutive representative days during the experiment (DOE 12 and DOE 13). Blue arrows indicate daily irrigation interventions carried out.

The spatio-temporal changing dynamics of AW and the contribution of each layer to the progressive lowering of AW in the whole soil profile during the days of the experiment is shown in Figure 9. The AW was preferentially depleted in the first soil layers (up to 30 cm), which showed the largest reductions, contributing to a reduction in AW contained in the whole soil profile ranging from 10% to 27% during the days of the experiment. The progressive reduction of AW in the first soil layers (up to 30 cm) was slightly more limited from DOE 13 when the vines increased their water uptake in the deeper layers (from 30 to 60 cm), contributing to an AW reduction in the whole soil profile in the range of 0% to 9% during the days of the experiment (Figure 9). In particular, the deeper layers showed no or small reductions in AW at DOE 2 and DOE 3, respectively. From DOE 13, soil water conditions also began to be limiting in the deeper layers (from 30 to 60 cm), as the vines gradually accessed AW after the superficial soil layers had exhausted the more freely available water.

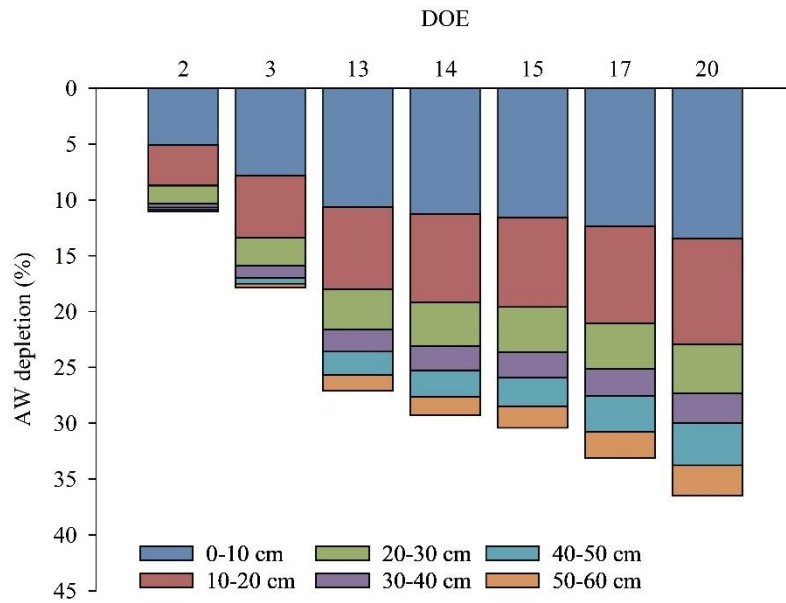


Figure 9 Dynamics of available water (AW) depletion in the whole soil profile (0-60 cm) reported for each 10 cm soil layer (0-10, 10-20, 20-30, 30-40, 40-50, 50-60 cm) during some representative days of the experiment (DOE).

Measurements of plant water status and stomatal conductance combined with soil observations and root water uptake dynamics during DOE 2 and DOE 3 are shown in Figure 10. Based on the daily dynamics of Ψ_{stem} and g_{sw} , DOE 2 was identified as the day before the onset of the water stress condition, since these parameters were almost the same in both C and WS vines (Figures 10A, B). During DOE 2, AW started to decrease in the first soil layers (up to 30 cm), with approximately 31%, 22% and 10% of AW being depleted in the 0-10, 10-20 and 20-30 cm soil layers respectively, while deeper layers showed insignificant decreases in AW (Figure 10C). Similarly, DOE 3 was identified as the day of water stress onset, as both g_{sw} and Ψ_{stem} of WS vines were different from those of C vines (Figures 10D, E). In particular, Ψ_{stem} of the WS vines was equal to that of the C vines during the early morning hours, while it decreased significantly at midday, reaching a value of approx. -1.0 MPa, due to the combination of a higher evaporative demand and a rapid decrease in SWC, suggesting that the reduced water availability exacerbated the midday depression phenomenon (Figures 10D, E). During DOE 3, AW was further reduced by approx. 16%, 11% and 5% compared to DOE 2 in the 0-10, 10-20 and 20-30 cm soil layers, respectively (Figure 10F).

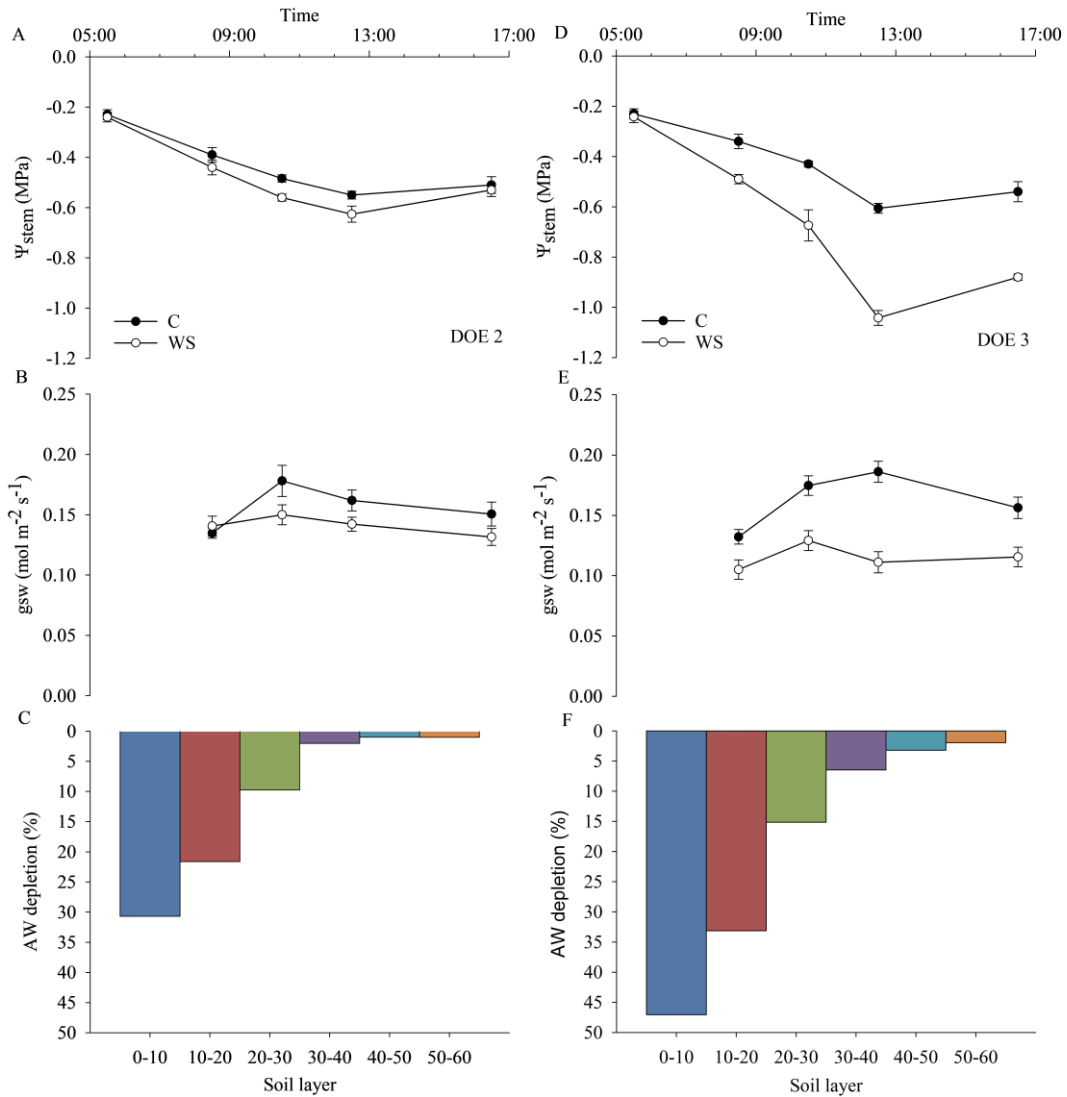


Figure 10 Daily dynamics of stem water potential (Ψ_{stem}), stomatal conductance (g_{sw}) and available water (AW) depletion calculated in relation to the content of each 10 cm soil layer (0-10, 10-20, 20-30, 30-40, 40-50 cm) during the day before (DOE 2) (A, B, C) and at the onset of water stress (DOE 3) (D, E, F).

2.4 Discussion

2.4.1 Plant behavior under non limiting soil water conditions

The irrigation strategy adopted for the well-watered vines consisted in splitting the daily irrigation volume into several interventions, maintaining the SWC steadily close to the FC (Figure 8A). The timely synchronization of the vine's water needs during the day, as influenced by environmental demands, with the irrigation supply, made it possible to guarantee an optimal soil water status at any time of the day, ensuring maximum root water uptake, avoiding the restriction of leaf gas exchange and the establishment of stomatal control and promoting transpiration processes (Figures 3B, C). This result is in agreement with Torres-Ruiz et al. (2016), who found that providing water

at times when environmental conditions are more stressful, i.e. during the hottest hours of the day, allows kiwifruit vines to benefit from an improved water status and higher leaf gas exchanges at this time of the day. Soil water status and plant transpiration rate, which is influenced by environmental evaporative demand, are two main factors influencing plant water status at a given time of the day (Tardieu and Simonneau, 1998) (Figures 3, 4, 7). Stem water potential measured in the present study was used as the primary indicator of plant water status because it has been shown to be less variable than leaf water potential and more efficient in detecting small differences between irrigation treatments (McCutchan and Shackel, 1992). A gradient exists between leaves and stem that is proportional to the transpiration rate, resulting in lower leaf water potentials than stem water potentials, as previously reported in kiwifruit by Morandi et al. (2010). Therefore, when interpreting the data, it should be taken into account whether the results refer to leaf or stem water potential values. Maximum values of Ψ_{stem} were observed at pre-dawn, when an equilibrium between plant and soil water potentials is achieved in the absence of water flux (Tardieu and Simonneau, 1998). Ψ_{stem} reached lower values as the environment became more water demanding (Figure 4), but well-irrigated vines maintained their Ψ_{midday} in the range between -0.4 and -0.7 MPa, below which vines began to experience a stress condition that significantly altered normal plant functioning. Previous studies reported daily trends in leaf water potential for well-irrigated 'Hayward' kiwifruit ranging from -0.1/-0.2 MPa at pre-dawn to -0.9/-1.2 MPa at midday (Gucci et al., 1997), -0.03/-0.08 MPa at pre-dawn to -0.4/-0.8 MPa at midday (Judd et al., 1989) and -0.6/-1.0 MPa at midday (Montanaro et al., 2009; Calderón-Orellana et al., 2021), with differences depending on environmental evaporative demand (i. e. VPD conditions), stomatal behavior and root system development.

2.4.1.1 Plant response to environmental demand

The plant functioning mechanism of the yellow-fleshed kiwifruit cultivar was first investigated by considering the relationships between Ψ_{stem} , g_{sw} and E with the variable climatic conditions expressed by VPD, with soil moisture as a non-limiting factor. The increasing trend in VPD associated with climate change is recognized as one of the main climatic drivers affecting plant physiology (Grossiord et al., 2020). Kiwifruit vines showed diurnal fluctuations in Ψ_{stem} in response to both evaporative demand (i.e. VPD) (Figure 3A) and light-induced stomatal opening (Figure 4) under non-limiting soil water conditions. Well-watered kiwifruit vines exhibited slight fluctuations in Ψ_{stem} during the day (Figure 4), which contribute to the low hydrostatic pressure gradient between leaves and roots in kiwifruit (Dichio et al., 2013), indicating low hydraulic resistances in the water-conducting pathway (McAneney and Judd, 1983). Ψ_{stem} declined with

increasingly warm and dry environmental conditions under non-limiting soil water conditions (Figure 3A), but it is expected to become less responsive to changes in VPD as soil water availability begins to decrease. Vine transpiration was positively correlated with VPD (Figure 3B), as previously observed (Montanaro et al., 2007), and increased with environmental demands (Grossiord et al., 2020), thus slightly reducing potentials to the minimum values allowed, generally recorded during the hottest hours of the day, without affecting physiological performances (Figure 4). The gsw of well-watered vines, unlike Ψ_{stem} and E, was not affected by the fluctuations in air VPD recorded during the days of the experiment, suggesting a plant behavior more likely to leave stomata open and allow high transpiration rates, which increased linearly with VPD, consequently lowering potentials (Figure 3C). A "midday depression" of gsw has been commonly reported in field-grown plants and attributed to high air VPD conditions (Tardieu and Simonneau, 1998). However, gsw reductions were generally not observed in well-watered plants, which showed almost constant gsw values despite high VPD, as previously described in kiwifruit (Gucci et al., 1996). This result is in agreement with Bardi et al. (2022), who studied the relationship between gsw and VPD in kiwifruit orchards (cv 'Hayward'), confirming a very low stomatal sensitivity of kiwifruit to VPD conditions and thus a tendency to anisohydric behavior. Stomatal sensitivity to air VPD affects the capacity of plants to regulate gas exchange in response to increasing VPD by controlling stomatal aperture as a strategy to decouple the canopy from the water-demanding atmosphere (Zweifel et al., 2007), and is highly variable between and within species (Grossiord et al., 2020).

2.4.2 Plant behavior under limiting soil water conditions

The response of yellow-fleshed kiwifruit to progressive SWC lowering was investigated to evaluate the effects of SWC on plant water status and physiological parameters and identify thresholds below which plant performance began to be compromised, up to severe effects.

2.4.2.1 Plant water status

Vines exposed to a progressive reduction in soil water availability had decreasing Ψ_{stem} values, revealing particularly severe stress conditions when significant differences from before dawn were observed. Ψ_{predawn} is usually used as a reliable indicator of plant water status (Judd et al., 1989), which is influenced by the maximum soil water potential to which roots are exposed. The progressive decrease in Ψ_{predawn} of WS vines indicated the inability of the plants to restore, during the nighttime hours, the tissue water reserves partially ceded to the transpiration flow during the day due to soil water shortage. The main physiological mechanisms of response to soil water deficit were reviewed, confirming that Ψ_{predawn} decreases in response to increasing soil water

deficit until the maximum level of water stress, identified as the time before irreversible damage and wilting of leaves occur, which was previously reported to range between pre-dawn leaf water potential values of -0.78 MPa and -1.06 MPa for green-fleshed kiwifruit (Gucci et al., 1997). A pre-dawn leaf water potential value of -1.0 MPa has been identified as the threshold below which vines are defined as 'severely stressed', with midday water potential values falling dramatically below those measured in well-watered vines (Judd et al., 1989; Gucci et al., 1997; Montanaro et al., 2007), while a value of -0.3 MPa has been identified as the threshold below which gsw begins to be affected (Gucci et al., 1996). Although plants subjected to decreasing soil water availability showed reduced Ψ_{predawn} , which can be used as a reliable indicator of the maximum soil water potential to which the roots are exposed (Tardieu and Simonneau, 1998), Ψ_{predawn} values are not always sufficient to detect water stress in kiwifruit, as differences in gsw can be found even when differences in water potentials are not appreciable. This was also evident in the current study, particularly on DOE 3, where Ψ_{stem} values were the same between C and WS vines at predawn, but differed significantly at midday (Figures 2, 10D), reflecting a condition of early mild stress, also supported by a reduction in gsw of approx. 40% at midday (Figure 10E). Therefore, Ψ_{midday} and gsw could be considered as more sensitive indicators of early stages of water stress in yellow-fleshed kiwifruit. This is in agreement with Gucci et al. (1996), who found that Ψ_{predawn} was insensitive to the initial phase of soil water content decrease and was significantly affected only after reaching the threshold value of -0.3 MPa.

2.4.2.2 Stomatal activity

Decreasing soil moisture induces plants to regulate stomatal conductance, in order to avoid damage to the hydraulic system, and the intensity of gsw reduction depends on plant stomatal behavior, ranging from anisohydric to isohydric strategies (Tardieu and Simonneau, 1998; Martínez-Vilalta et al., 2014; Grossiord et al., 2020). Whether kiwifruit vines have an isohydric or anisohydric behavior is still highly discussed, but it is increasingly accepted that isohydricity is cultivar rather than species specific, and is also influenced by external conditions and the peculiar environments in which plants grow. Previous studies have found that gsw of 'Hayward' kiwifruit is reduced by stomatal closure under severe water stress (Chartzoulakis et al., 1993; Gucci et al., 1996). Gucci et al. (1997) found smaller differences between leaf water potentials of stressed and well-irrigated vines when measured at midday than at pre-dawn, supporting the conclusion that 'Hayward' kiwifruit responds conservatively to soil moisture depletion by closing stomata, limiting transpiration and thus the decline in leaf water potential at midday (tendency to isohydric behavior), which is not in agreement with other research that suggest a lack of or poor stomatal

control of transpiration, with leaves continuing to lose water as soil water deficit increases (Judd et al., 1989; Calderón-Orellana et al., 2021). In particular, Montanaro et al. (2007) suggested that 'Hayward' kiwifruit can cope with the Mediterranean environment by adopting an adaptive strategy aimed at low gsw and carbon gain sacrifice for water conservation and photoprotection, also under severe water stress, disapproving the absence of stomatal control. From the measurements carried out in the present study, the differences between Ψ_{stem} of C and WS vines were greater at midday (Figure 4), suggesting that yellow-fleshed kiwifruit tends not to have the same stomatal behavior as that found by Gucci et al. (1997) in green kiwifruit, still slightly increasing E during the hottest hours of the day and significantly decreasing potentials, although there was a consistent reduction in gsw of WS vines, which was only 25-44% of that of C vines throughout the experimental trial (Figure 4), in agreement with Gucci et al. (1997). Therefore, the gsw of yellow-fleshed kiwifruit vines appeared to be strongly affected by changes in soil water status (Figure 6B), in agreement with Gucci et al. (1996), identifying SWC as a key driving variable involved in the behavior of kiwifruit vine stomata. Stomatal behavior of kiwifruit has been reported to be highly sensitive to soil and air hydration, contributing to minimize leaf water deficits and regulate overall canopy transpiration (Gucci et al., 1996). An average value of gsw reduced by 65% compared to C vines was achieved when Ψ_{midday} was lower than -0.8 MPa (Figure 5A), confirming the high stomatal sensitivity to changes in plant water status, as previously reported by Gucci et al. (1996). The relationship between gsw and Ψ_{midday} suggests that the stomatal response occurs after a threshold value of plant water potential is reached (Garnier and Berger, 1987), showing a progressive closure of stomata when Ψ_{stem} falls below the critical value (Figure 5A). This result, obtained under field conditions, is consistent with the finding of Mills et al. (2009), who investigated the water stress physiology of pot-grown yellow-fleshed kiwifruit and identified a midday leaf water potential value of approx. -1.0 MPa as the threshold below which gsw began to be significantly affected (reduced by approx. 80% compared to control vines when leaf water potential reached values of -1.5 MPa). At the same time, it does not fully agree with Boini et al. (2022), who identified -0.5 MPa as the midday stem water potential value of field-grown vines below which *A. chinensis* var. *chinensis* reduces all leaf gas exchange parameters. Several research studies agreed that A and gsw of field-grown vines follow trends parallel to those of container-grown vines, but the stress develops more gradually when irrigation is restricted in the field (Gucci et al., 1996), also due to the generally lower unsaturated hydraulic conductivity of pot substrates compared to natural fields (Tardieu and Simonneau, 1998). It is expected that under field conditions, the severity of water stress can be mitigated and vines perform better due to the more extensive and developed root

system, which explores a larger soil volume and compensates for the effects of water stress by absorbing water from greater soil depths (Goldhamer et al., 1999; Mills et al., 2009).

2.4.2.3 Sensitivity to drought stress and stem water potential threshold

The diurnal changes observed in leaf E reflected the changes in gsw, suggesting that stomatal closure was an adaptive strategy to prevent leaf dehydration by slowing transpiration (Figure 4). Despite the relatively early adaptive response of stomata, which limits further decreases in water potential and ensures that leaf water demand does not exceed the supply capacity of the hydraulic system (Martin-StPaul et al., 2017), Ψ_{stem} was not protected and decreased significantly, probably due to the increased hydraulic resistance of progressively drying roots as reported by Gucci et al. (1996). The reduction in Ψ_{stem} was related to the progressive AW depletion (Figure 7A) and could therefore be dependent on the increased root hydraulic resistance. Soil drying has been reported to induce changes in root hydraulic conductance, which decreased rapidly but reversibly upon re-watering (Vandeleur et al., 2009; Black et al., 2012). In well-watered kiwifruit vines T_{leaf} was higher than air temperature, with large differences indicating a reduced capacity for evaporative water loss as previously reported in 'Hayward' kiwifruit (Buwalda et al., 1992; Montanaro et al., 2007). However, ΔT decreased with increasing environmental demand (i.e. VPD) due to higher transpiration rates, resulting in increased evaporative water loss and cooling effect observed in kiwifruit vines under non-limiting soil water conditions (Figure 3D). Reduced transpiration induced by water shortage compromised the thermoregulatory capacity of leaves, leading to a further increase in leaf temperature, approx. 2 to 4 °C higher than kiwifruit vines under non-limiting soil water conditions (Figure 5B). In kiwifruit, the small gradient between canopy and soil water potentials probably decreases water uptake, effectively reducing the ability of this species to tolerate drought (Gucci et al., 1996). Hydraulic conductivities measured for both root and stem xylem tissues are higher compared to other species, and the resistance to water movement within the vine is very low (McAneney and Judd, 1983; Dichio et al., 2013). This supports that the soil to root pathway is the main resistance to water uptake by the kiwifruit root system, even at high soil water potentials, which can be worsened by soil drying (McAneney and Judd, 1983). Although high whole-plant hydraulic conductance in kiwifruit contributes to high transpiration rate and photosynthetic capacity, it can also lead to increased susceptibility to some drought-related hydraulic impairments (e.g. cavitation, embolism), which are exacerbated during drought and in Mediterranean environments (Dichio et al., 2013; Bardi et al., 2022). Most of the studies on kiwifruit water relations and needs refer to *A. chinensis* var. *deliciosa*, while information on the physiology of yellow-fleshed kiwifruit under non-limiting soil water conditions and reduced soil

water availability is emerging recently. The results obtained in the present work show a change in the behavior of kiwifruit vines exposed to different soil water conditions. In particular, kiwifruit vines moved from a high water-consuming approach, observed under non-limiting soil water conditions, without limiting their activities or suffering from critical environmental conditions (e.g. high VPD), to a more conservative adaptive strategy adopted under conditions of limited soil water availability. The current study suggests the identification of a threshold value of the plant water status, influenced by different soil water conditions, which can be considered critical for *A. chinensis* var. *chinensis*, i.e. Ψ_{midday} values below -0.8 MPa indicated an initial impairment of leaf gas exchange activity (Figure 5A). From the measurements carried out in the present study, the Ψ_{stem} variations of WS vines from the control values during DOE 3, reaching a value of -1.0 MPa at midday, identify the establishment of a stress condition. Instead, Ψ_{predawn} and Ψ_{midday} values of -1.0 and -1.8 MPa, respectively, corresponding to the maximum level of water stress achieved during the experimental trial, already identify a severe water stress condition in yellow-fleshed kiwifruit. Threshold values reported for *A. chinensis* var. *chinensis*, below which physiology appeared to be compromised, were higher than those reported for *A. chinensis* var. *deliciosa* in a typical Mediterranean environment (Montanaro et al., 2009), supporting the lower tolerance of yellow-fleshed kiwifruit to reduced water availability compared to green-fleshed kiwifruit, cv 'Hayward'. This is consistent with the findings of Rahman et al. (2011), who demonstrated that *A. chinensis* var. *deliciosa* had a higher water loss than *A. chinensis* var. *chinensis* during a mild or short-term period of water stress, but a higher tolerance and then greater advantages under conditions of severe or prolonged water stress.

2.4.3 Spatio-temporal dynamics of soil moisture and thresholds for precision irrigation

Monitoring soil water status, which is related to plant water status, is increasingly becoming a common approach to schedule and control irrigation in order to ensure optimal plant performances (Nolz et al., 2016). Fluctuations in soil AW vary throughout the soil profile, depending on the structure and development of the root system, the irrigation system wetting a specific soil volume, and the environmental conditions (such as reference evapotranspiration and VPD). Therefore, it is important to define in-situ soil moisture thresholds based on multi-layer sensor data collected in the field to manage irrigation from a practical point of view. The method proposed in this paper defines thresholds for AW by measuring plant responses to progressive lowering of SWC and analyzing their relationships, in order to identify the 'stress point' that serves to early detect a water stress condition and implement more sustainable and precise irrigation strategies. The present study found that plant water status and other physiological indicators of kiwifruit vines were

sensitive to a progressive decrease in SWC (Figure 6). This result is consistent with the findings of previous research carried out on other species (Garnier and Berger, 1987; Naor and Cohen, 2003; Al-Yahyai, 2012; Jamshidi et al., 2020), focusing on the response of water stress indicators to soil drying and whether these parameters are correlated with each other. The resulting correlation confirmed that, in addition to environmental variables, other factors such as soil moisture could have a great influence on plant water status. In the lower range of SWC, plant water status and physiological parameters were controlled by the root water uptake mechanism and water availability. The current work aimed to identify soil water content thresholds and define the amount of AW that can be used by the plant without compromising the physiological aspects, delimiting the range where no stress should occur. Results provide new information to define the depletion fraction of AW from the root zone before moisture stress occurs in yellow-fleshed kiwifruit, which was previously considered to be 0.35 for kiwifruit (cv 'Hayward') with a crop evapotranspiration (ET_c) of approx. 5 mm day^{-1} (Allen et al., 1998). Vines, which absorbed water differentially in each soil layer, experienced a decrease in the AW amount of approx. $35 \text{ m}^3 \text{ ha}^{-1}$ from the soil profile achieved at DOE 2, identified as the day until which the plant's water status and physiology were maintained at optimal levels, which corresponds to a depletion of about 11% of the AW contained in the whole soil profile (0-60 cm) (Figure 9). Instead, AW was overall reduced by approx. 17.8% at DOE 3, identified as the 'stress onset day' (Figures 10D, E), which implied a decrease of about $57 \text{ m}^3 \text{ ha}^{-1}$ of AW from the whole soil profile (Figure 9). The depletion factor changes with the environmental evaporative demand, in particular at high ET_c it is 10-25% lower than the ordinary value, indicating that a lower AW fraction can be depleted before the onset of the stress (Allen et al., 1998). Values found in the present study highlighted that under the same ET_c conditions, the depletion factor of AW for yellow-fleshed kiwifruit was approx. 50% lower than that reported for 'Hayward' in the FAO-56 paper. A previous study has focused on defining the allowable soil water depletion for precise irrigation scheduling in other tree crops, and obtained a depletion factor lower than those tabulated in the FAO-56 paper (Puig-Sirera et al., 2021). The assessment of root water uptake through changes in soil moisture is promising (Jackisch et al., 2020) and provides further information on the amount of water depleted, which can be considered complementary in estimating transpiration and water needs of plants. The greater activity of surface roots is shown by the rapidly changing pattern of both SWC around the C vines and AW depletion around the WS vines, which followed a decreasing trend with increasing soil depth (Figure 8). This is consistent with previous research investigating the pattern of SWC at different depths in the root zone of kiwifruit vines, which showed that vines preferentially use near-surface water when available (Clothier and Green, 1994; Green and Clothier, 1995; Green et al., 2006).

Preferential root water uptake occurred initially from the superficial, wetter parts of the soil, and then increased in the deeper layers. Vines began to extract water from the depths where water was more freely available (Figure 9), in accordance with Green and Clothier (1995) who confirmed the capacity of kiwifruit vines to shift their water uptake pattern in response to changes in soil water availability. In the present study, soil water depletion was used to understand the intensity and dynamics of root water uptake throughout the root zone. Considering the dynamics of root water uptake, soil moisture thresholds should be identified for each layer, which contribute differently to triggering water stress. Therefore, in Mediterranean environments where daily vine water requirement can reach about $60\text{--}70\text{ m}^3\text{ ha}^{-1}$ during the hottest days, it is advisable to split the daily irrigation volume in two or more interventions, according to the DOE 2 depletion indication. Ensuring RAW throughout the day in the first 0–30 cm soil layer, mainly interested by root water uptake, is essential to avoid the establishment of water stress and in particular of midday depression phenomena.

2.5 Conclusions

The analysis of stem water potential and leaf gas exchange of yellow-fleshed kiwifruit showed that the atmospheric condition (VPD) did not affect stomatal closure/opening under non-limiting soil water conditions, indicating that vines do not implement stomatal control preferring to transpire, losing water according to its availability and thus lowering the potentials. Yellow-fleshed kiwifruit is then characterized by a high water-consuming behavior when soil water availability is not limiting, outlined in a Mediterranean environment. However, stomata appeared to be sensitive to changes in soil water status, decreasing their conductance as soil water content progressively declined, representing a protective and adaptive strategy to water deficit. The approach based on both soil water monitoring and determination of plant physiological parameters was used to identify soil moisture thresholds of high practical applicability for irrigation management. Monitoring of stem water potential and stomatal activity throughout the experiment made it possible to identify the point before and at the onset of water stress, below which plant water status and physiological activity began to be affected, and to define in situ irrigation thresholds. The results of the study suggest that the AW that can be depleted before water stress occurs is approximately 11% of the AW of the 0–60 cm soil layer, which appears to be lower than the standard value reported in the FAO-56 paper. The spatio-temporal dynamics of root water uptake highlighted a different contribution of each soil layer to meeting the transpiration demands of the vines. The decrease in AW was more pronounced in the superficial soil layers (i.e. 0–30 cm), which are mainly affected by root water uptake, contribute more to the onset of stress and should

therefore be carefully managed by irrigation. Although the experimental trial was carried out in a semi-arid Mediterranean environment, the approach can be transferred to other environments. Future research is needed to define and validate information on the stress coefficient (K_s) and the AW depletion fraction throughout the irrigation season and in different soil and environmental conditions, in order to develop precision irrigation strategies that allow plants to meet transpiration demand and prevent the establishment of temporary water stress conditions, which are particularly common in Mediterranean-type climates.

2.6 References

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3. Combining plant-based sensing and mechanistic modelling to quantify hydraulic resistance and capacitance for real-time irrigation in Mediterranean yellow-fleshed kiwifruit orchards

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Abstract

Climate change and increasing water scarcity, especially in the Mediterranean region, are major challenges for modern agriculture, requiring the implementation of real-time irrigation management methods to improve whole-plant water use efficiency. In the present study, two plant-based measurements (trunk sap flow and trunk water potential, ψ_{trunk}) were used in combination with a dynamic water flow model to estimate hydraulic capacitance (C) and hydraulic resistance (R) along the water transport pathway in yellow-fleshed kiwifruit vines grown in a Mediterranean environment under both well-watered and drought conditions. A sensitivity analysis of the model was performed to select a subset of identifiable parameters, accounting for most of the variability in model predictions of ψ_{trunk} . Based on the identifiable parameters, two model calibrations were performed: (1) model calibration of C and R; (2) model calibration of C, R and the initial amount of water stored in the stem compartment. These parameters were recalibrated daily based on a 1-day moving window. The best model performance under soil water limiting conditions was achieved when all three parameters were used for calibration. C and R parameters strongly correlated with ψ_{trunk} , revealing the hydraulic behavior and drought response of kiwifruit vines. In particular, C was found to decrease with more negative ψ_{trunk} values, whereas R showed an increase. In addition, C and R varied within a narrow range of ψ_{trunk} fluctuations, as occurred in well-watered vines. While the proposed modelling approach requires investment in sensor technologies and a data management and modelling platform, it offers the potential to quantify and visualize daily dynamics in plant hydraulic parameters and provide farmers with valuable tools to improve real-time irrigation management of irrigation in the era of precision agriculture.

3.1 Introduction

In the current climate change scenario, the Mediterranean region is recognized as one of the world's water crisis hot spots due to significant increases in temperatures and decreases in rainfall (with fewer events but of greater intensity), threatening water availability throughout the region (Milano et al., 2013; Lionello and Scarascia, 2018; Prada et al., 2024). Climate change affects crop phenological stages and irrigation water requirement, and increases the frequency of extreme weather events, such as droughts and heat waves (Soares et al., 2022). In this specific climatic context, kiwifruit needs to face high temperatures, irradiance and leaf-to-air vapor pressure deficit during summers (Montanaro et al., 2009). In particular, the kiwifruit species belongs to the lianas and has developed peculiar morpho-anatomical traits and hydraulic system features adapted to its native habitats, which are characterized by moderate light intensity and abundant rainfall (1200-1800 mm year⁻¹) (Ferguson, 1984), ensuring non-limiting water conditions. Sparse and large xylem vessels (200-350 µm in diameter) contribute to low xylem hydraulic resistance to vertical water flow and allow large amounts of water to be transported upwards, with very high stem hydraulic conductivity (Dichio et al., 2013). Large leaves, which provide a wide transpiring surface, contribute to high daily transpiration rates, reaching approximately 40-60 L day⁻¹ plant⁻¹ in a Mediterranean kiwifruit orchard (Dichio et al., 2013; Green et al., 2022; Xiloyannis et al., 2023). The vascular tissue is composed of semi-ring porous wood, which contributes to low axial xylem resistance (Condon, 1992). The high leaf-specific whole plant conductance (i.e., whole-plant conductance normalized by leaf area, Dichio et al., 2013) of kiwifruit vines compared to olive plants, which is attributable to the larger conducting vessels, could contribute to the susceptibility of this species to embolism formation under drought conditions (Dichio et al., 2013), generally exacerbated by high temperatures and high evaporative demand. Clearwater and Clark (2003) reported that embolism formation occurs more frequently when xylem tensions are high, and less frequently when drought stress is relieved. They also found that embolism formation occurs more frequently and at lower xylem tensions in *Actinidia* vines than in *Ripogonium*. In-depth studies of water flow dynamics, plant water status and drought susceptibility have become urgent challenges that need to be addressed in the current climate change scenario, as more extreme drought events are expected to occur in the coming years (Da Sois et al., 2024) and irrigation water supply cannot be guaranteed at all times. As soil water content decreases, hydraulic resistance to water transport through the soil increases and soil matric potential decreases, creating adjusted water potential gradients within the plant that drive water flow (Scharwies and Dinneny, 2019). As plants respond differently to water deficit (Scharwies and Dinneny, 2019), information on the plant hydraulic resistance encountered in the soil-plant continuum and its changes due to drought

stress conditions is important as it strongly influences plant water status and helps to understand important plant processes occurring under these conditions (Baert et al., 2015).

The high water requirements of kiwifruit (Chartzoulakis et al., 1993; Holzapfel et al., 2000; Calderón-Orellana et al., 2021) and its susceptibility to water deficits (Judd et al., 1989; Montanaro et al., 2007; Mills et al., 2009; Calderón-Orellana et al., 2021) highlight the importance of effectively and properly quantifying irrigation requirements and identifying water stress indicators and thresholds. Proper irrigation management helps to mitigate the adverse effects of environmental stressors caused by climate change (Rajan et al., 2024). It limits midday depression in physiological parameters, such as photosynthetic rate, and sunburn of fruits and leaves, which are exacerbated by increasing temperatures and radiation during the hottest hours of the day (Gucci et al., 1996; Montanaro et al., 2009; Wu et al., 2023) and pose a threat to kiwifruit production in Mediterranean environments. The implementation of innovative plant-based irrigation management methods can be of great importance to kiwifruit contributing to a more precise irrigation for this crop. Precision irrigation, driven by data on soil water status, plant responses and weather variables, delivers water at the right time and in the right amount, contributing to water saving, improvement of whole-plant water use efficiency and timely fulfilment of crop water needs (Bwambale et al., 2022). Furthermore, delivering the right amount of irrigation water to meet the crop's needs at different times, also limits the onset of transient drought stress (Brahmanand and Singh, 2022), which can easily occur throughout the day especially in arid and semi-arid environments. Several soil and plant sensors have been suggested as effective irrigation tools, and trunk diameter variations, sap flow and plant water potential are the most commonly used plant sensors in scientific studies (Moriana et al., 2012). Plant water status monitoring, integrated with soil and environmental information, can support precision irrigation and inform irrigation scheduling (McCutchan and Shackel, 1992; Goldhamer et al., 1999; Intrigliolo and Castel, 2004; Ortuño et al., 2010; Shackel, 2011). Plant physiological and drought stress indicators are therefore increasingly accepted and used by farmers as useful diagnostic tools (Fereres and Evans, 2006; Noun et al., 2022).

Despite significant advancements in soil- and plant-based sensors, which enable continuous monitoring and provide real-time feedback on soil and plant water status, enhancing our understanding of plant responses to different soil and environmental conditions and enabling more accurate irrigation management, the widespread adoption of effective real-time decision support methods for irrigation is still lacking. A significant gap between current irrigation practices and what is considered best practice in most cases is observed and remains a major concern (Fereres and Evans, 2006).

This gap can be filled by integrating plant measurements and mechanistic modelling to develop novel plant-based irrigation scheduling methods (Steppe et al., 2008; Steppe, 2013). Although such models are already available in the scientific literature and thoroughly validated (Steppe et al., 2006, 2008; De Pauw et al., 2008a), their practical application in irrigation scheduling has not yet been fully exploited. Moreover, modelling plant water transport dynamics during drought stress can deepen our understanding of the underlying processes and mechanisms plants use under such conditions (Steppe et al., 2008; Baert et al., 2015; De Swaef et al., 2022). This knowledge is particularly important in the climatically challenging Mediterranean region, where it can help assess the impacts of climate change on crop performance.

The present study integrates continuous plant measurements from sensors, such as trunk sap flow and trunk microtensiometers, with a mechanistic water flow model to achieve the following objectives: (i) simulate trunk water potential dynamics (ψ_{trunk}) to predict changes in hydraulic capacitance (C) and resistance (R) of yellow-fleshed kiwifruit vines under well-watered and drought conditions in a Mediterranean climate; (ii) provide practical insights into model calibration for more accurate estimation of key hydraulic parameters, especially under drought conditions; and (iii) illustrate the potential of the mechanistic model as an effective tool for plant-based, real-time irrigation scheduling in fruit crops.

3.2 Materials and methods

3.2.1 Plant material and experimental site

The experiment was carried out during the 2023 growing season in a commercial and mature kiwifruit orchard (*Actinidia chinensis* var. *chinensis*, cv. Zesy002) planted in 2014 in Metaponto (South Italy, 40°24'N 16°46'E) over a 4-week period from August 2 till 15 September (from Day-of-the Year (DOY) 214 until DOY 258). The yellow-fleshed kiwifruit cultivar was grafted onto D1 rootstock (*A. chinensis* var. *deliciosa*), cultivated at 5 m x 2 m spacing and trained as a pergola system. The soil profile consisted of a uniform sandy clay loam texture (58% sand, 14% silt and 28% clay). The experimental plot consisted of two irrigation blocks equipped with independent valves and analogue flow meters. A manual flow meter (TEV58R GU-SD, Greiner S.p.A., Italy) was used to record the irrigation volumes distributed and an automatic irrigation controller (“Irrifarm” system, Pan. Agri S.r.l., Italy) was used to remotely control the water supply. Irrigation was provided by micro-sprinklers with a 5 m x 1 m spacing in the vine row, using a single auto-pressure compensating emitter per vine at a flow rate of 40 L h⁻¹ and a wetted radius of 0.9 m.

3.2.2 Irrigation scheduling and treatments

All vines were grown under optimal soil water availability conditions throughout the season until the beginning of the experiment. Daily vine water requirements (crop evapotranspiration, ET_c) were estimated using reference evapotranspiration (ET_0) and crop coefficient (K_c) (Allen et al., 1998), with K_c ranging from 0.5 to 1.1 along the season (Steduto et al., 2012). The irrigation requirement was calculated using the following equation:

$$IR = (ET_c - ER)/ime \quad (1)$$

where IR is the irrigation requirement (mm), ET_c is the crop evapotranspiration (mm), ER is the effective rainfall [calculated by considering daily rainfall < 5 mm as ineffective (Dastane, 1974)], and ime is the irrigation method efficiency (0.95). The soil volume wetted by irrigation was calculated considering the sprinkler wetting radius, the depth of the wetting pattern (0.6 m) and the number of emitters per hectare. The irrigation requirement was adjusted weekly based on continuous monitoring of the water content by soil moisture probes. Real applied irrigation volumes were derived from increases or decreases in irrigation requirement in order to maintain the soil moisture level of the soil volume wetted by irrigation within field capacity (FC) and readily available water (RAW) (Mininni et al., 2022; Calabritto et al., 2024). Two irrigation treatments were applied: a well-watered irrigation treatment, in which irrigation returned 100 % of irrigation volume defined according to the vines' water need and soil moisture monitoring, and a drought irrigation treatment, in which irrigation was reduced to develop progressive levels of drought stress. In particular, the well-watered irrigation treatment received daily irrigation volumes of 45 m³ ha⁻¹ (DOY 214-216; DOY 222-229; DOY 241-253) and 60 m³ ha⁻¹ (DOY 217-221; DOY 230-240; DOY 254-258) throughout the experimental period. Daily irrigation volumes were split into three/four irrigation events throughout the experiment, except for the period from DOY 220 to DOY 237, where irrigation volumes were split into only one or maximum two events per day (early in the morning and late in the afternoon). In contrast, in the drought treatment, irrigation was first ceased (DOY 220-226) and then reduced (DOY 227-236, 15 m³ ha⁻¹) with full re-watering on DOY 237, and then ceased again (DOY 244-255) with full re-watering on DOY 256. In the drought irrigation treatment, daily irrigation volumes were distributed in a single intervention from DOY 227 to DOY 237.

3.2.3 Environmental and soil measurements

Environmental variables, such as daily minimum, maximum and mean air temperature, relative humidity and net solar radiation were recorded by a regional meteorological station located about 1 km from the experimental field. Hourly air vapor pressure deficit (VPD) was calculated using hourly values of air temperature and relative humidity (Goudriaan and van Laar 1994). ET_0 was calculated using the Penman-Monteith equation (Allen et al., 1998).

Frequency domain reflectometry (FDR) 90 cm multi-profile soil moisture probes (Drill & Drop, Sentek Sensor Technologies, Stepney, Australia) were used to continuously monitor the volumetric soil water content (θ , $\text{cm}^3 \text{cm}^{-3}$) in 10 cm increments throughout the soil vertical profile, allowing the remote detection of instantaneous oscillations in each soil layer. A soil layer of 0-60 cm was monitored in the present study, as this depth has been previously reported to be useful for irrigation purposes in kiwifruit based on soil type and root development and uptake activity (Xiloyannis et al., 2023; Calabritto et al., 2024). The probes were installed in the row at a distance of 0.5 m from the vine trunk and approximately in the center of the wet soil volume.

The relation between θ and the matric potential (h) was investigated through the water retention curve obtained with the Richards' pressure plates apparatus (Richards, 1948) in the 0-60 cm soil profile. Hydraulic characterization of the soil was conducted by fitting measurement data to the van Genuchten equation (1980) (Fig. 1).

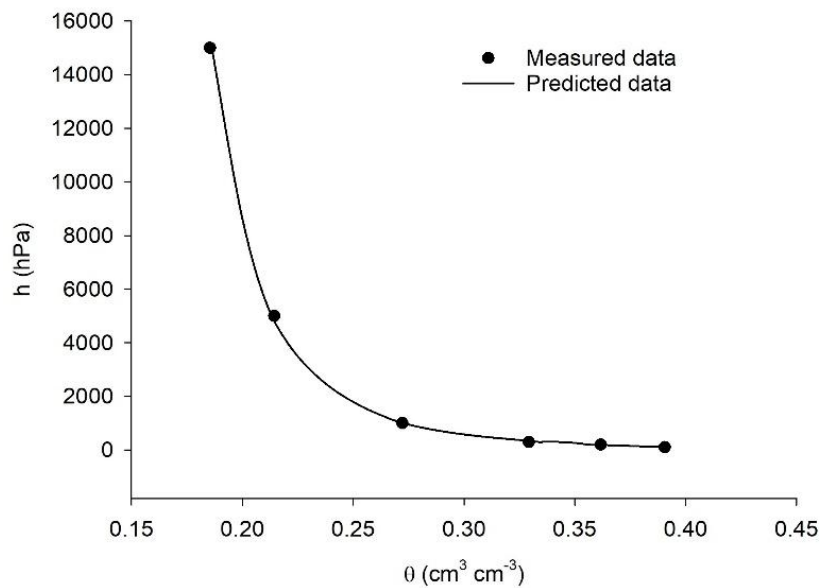


Fig. 1. Water retention curve of the sandy clay loam soil profile (0-60 cm). The van Genuchten function was fitted to the water retention data.

Based on estimated parameter values (Table 1), the matric potential of the soil (ψ_{soil} , MPa) can be calculated using the equation of van Genuchten (1980):

$$\psi_{\text{soil}} = \frac{1}{\alpha} \left[\left(\frac{\theta - \theta_r}{\theta_s - \theta_r} \right)^{\left(\frac{1}{m} \right)} - 1 \right]^{\frac{1}{n}}$$

where θ_r and θ_s are the residual and saturated volumetric water content ($\text{cm}^3 \text{cm}^{-3}$), respectively, α (cm^{-1}), n (dimensionless) and $m = 1 - 1/n$ (Mualem, 1976) the form parameters.

Table 1 Estimated parameters of the van Genuchten (1980) equation for the studied kiwi orchard.

Parameter	Estimation
θ_s ($\text{cm}^3 \text{cm}^{-3}$)	0.3974
θ_r ($\text{cm}^3 \text{cm}^{-3}$)	0.1891
α (cm^{-1})	0.0115
n (-)	1.3086
m (-)	0.2358

3.2.4 Plant sensor measurements

Trunk water potential (ψ_{trunk} , MPa) of kiwifruit vines was continuously monitored by microtensiometers (FloraPulse, Davis, CA, USA) embedded directly in the trunk. The sensors were installed at pre-dawn and reached equilibrium with the vine xylem within a few days after installation (Pagay, 2022). Data of ψ_{trunk} were collected at 20 min intervals, stored in a memory card inserted into the solar powered wireless data logger and uploaded to a cloud repository. Trunk microtensiometers were installed in the field on two vines for each irrigation treatment (well-watered and drought).

Trunk sap flow (SF, kg h^{-1}) was measured in four vines for each irrigation treatment. The selected vines had straight trunk sections and showed uniform canopy dimensions. Each vine was instrumented with a single set of three-channel sensors (model HP3TC, Tranzflo NZ Ltd, Palmerston North, New Zealand). The probes were installed in each trunk at a height of approximately 0.75 m above the ground. One temperature probe (the main sensor) and a second probe (the reference sensor) were positioned 15 mm and 40 mm, respectively, downstream of the heater probe. The vine trunk close to the sensors was wrapped in aluminum foil for thermal and light insulation. Sap flux density was measured using the T-max heat-pulse method (Cohen et al.,

1981; Green et al., 1989, 2003) with a slight change in sensor spacing (5 mm larger distance between the heater and the sensor) to cope with the very high sap flux densities ($J_s < 200 \text{ cm h}^{-1}$) expected in kiwifruit vines during the middle of the day. The wider sensor spacing also improves the resolution of low flow rates down to about 1 cm h^{-1} (Green et al., 2003). A data logger (model CR1000, Campbell Scientific, Logan, USA) was used to check the heater output and to measure the time of maximum temperature rise after the application of a short (4 s) heat pulse. Sap flux densities were calculated from the time values of maximum temperature rise, including a wound correction factor to account for the effect of wounding caused by the probes (a wound diameter of 2.8 mm was assumed for the 2.0 mm diameter drill holes; Green et al. (2022)). Data of SF were collected at 30 min intervals.

3.2.5 Model description

An existing mechanistic water flow and storage model (Steppe et al., 2006, 2008), which describes water transport dynamics in individual plants, was adapted in the present study (Fig. 2).

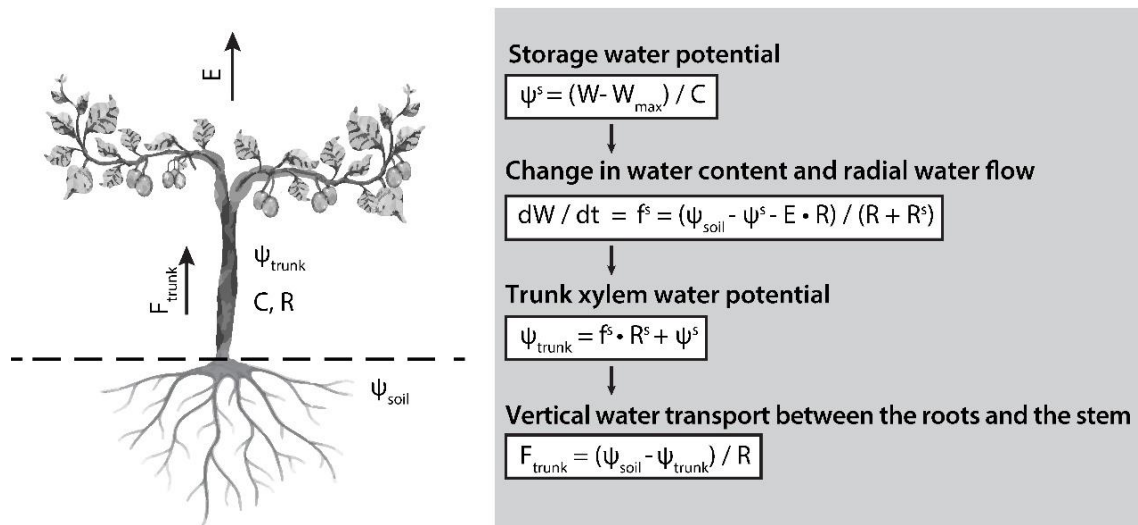


Fig. 2. Schematic representation and mathematical equations of the dynamic water flow and storage model from Steppe et al., 2008. The model consists of an internal water storage compartment (stem) and external water source (soil) and a flow path (F_{trunk}). The model is driven by transpiration (E) and soil water potential (ψ_{soil}) to determine the hydraulic capacitance of the stem storage compartment (C) and xylem hydraulic resistance (R). Abbreviations in the scheme: W = water content in the storage compartment; $W_{\max}$ = maximum water content in the storage compartment (38 kg); R^s = hydraulic resistance of the storage compartment ($0.01 \text{ MPa h kg}^{-1}$); ψ_{trunk} = trunk water potential; ψ^s = stem storage potential; f^s = radial water flow.

The basic principles of the original water flow and storage model have been maintained to simulate changes in the ψ_{trunk} of a group of kiwifruit vines for each irrigation treatment. Continuously measured sap flow in the trunk (SF) and soil matric potential (ψ_{soil}), which is considered as a limiting external water storage pool, were used as inputs to the model. The model, which was simplified to describe the processes occurring in the stem compartment, consisted of a stem storage compartment (i.e., a stem storage pool including the living cells of the stem bark) and a vertical xylem flow path (i.e., F_{trunk}). The stem storage compartment was characterized by its hydraulic capacitance (C), which is defined as the ratio of the change in the amount of water present in the stem storage tissue (W) to the change in water potential of the tissue (Steppe et al., 2006). As transpiration starts in the morning ψ_{trunk} decreases, establishing a water potential gradient between stem and soil which induces water uptake from the soil. The developed water potential difference along the non-living xylem vessels forces water to move upwards, inducing a vertical water transport (Steppe et al., 2006; De Pauw et al., 2008a). Since the living tissues of the stem storage pool and the xylem are hydraulically connected, the water potential difference between the xylem and the bark induces a radial water flow allowing internally stored water to contribute to the transpiration stream (Genard et al., 2001; Steppe et al., 2006; Steppe et al., 2015a). As a result of the radial water flow, W varies. Furthermore, as water ascends, it encounters resistance in the form of hydraulic flow resistance within the xylem. The model developed by Baert et al. (2015) incorporates a variable integrated hydraulic resistance that captures the entire water transport pathway from soil to stem, which accounts for changes in overall hydraulic resistance in the soil-to-stem segment and is related to ψ_{soil} . In our study, the adapted model integrates the resistances of the soil, roots and trunk into a single parameter, R .

3.2.6 Model calibration and simulation

Model simulation, calibration and identifiability analysis were performed using the plant modelling software PhytoSim (Plant AnalytiX, Mariakerke, Belgium) for each irrigation treatment. All model equations were solved using a fourth order Runge Kutta fixed step size integrator (fixed step size = 0.1 h). Model calibration was based on the Shuffled Complex Evolution (global search method) and used to minimize the weighted sum of squared errors between the model output and the measured data for ψ_{trunk} . Continuous measurements of ψ_{trunk} were used as calibration variable. An identifiability analysis of the model was performed to select a subset of identifiable parameters (De Pauw et al., 2008a). A model parameter subset is considered identifiable when the collinearity index remains below 15, indicating limited linear dependency among the parameters, and the parameters demonstrate high influence on the model output. While

model output sensitivity lacks a definitive threshold, it serves as a useful ranking criterion for comparing potentially identifiable parameter subsets, and can help selecting the most appropriate subset (De Pauw et al. 2008a). Three identifiable key model parameters (i.e., calibration parameters) were found to influence ψ_{trunk} : the capacitance of the water storage tissue in the stem compartment (C), the hydraulic xylem resistance (R) and the initial value of the water content in the stem storage tissue (W). The remaining non-identifiable model parameters were set at a fixed value taken from the literature or directly calculated. Two types of model calibration were performed using the two distinct datasets, representing the well-watered and the drought irrigation treatments. Simulations of ψ_{trunk} were performed with only two calibration parameters (C and R) and with all three calibration parameters (C, R and initial W) for both irrigation treatments to compare the simulated pattern of ψ_{trunk} and analyze differences in the calibrated parameters. First model calibration was performed on DOY 214. The identifiable parameters were recalibrated daily based on a 1-day moving window (Steppe et al., 2008), which shifts daily using both initial conditions and parameters from the previous calibration for subsequent model simulations. This ensured a good model fit at all times, allowing investigation of the daily variability of the calibrated parameters in response to changes in ψ_{trunk} .

3.3 Results

3.3.1 ψ_{trunk} simulated with fixed or calibrated initial W

The experimental days were characterized by generally sunny and dry weather with high evaporative demand. VPD was quite variable throughout the experimental days, reaching maximum hourly values ranging from 1.85 kPa to 5.0 kPa observed on DOY 216 and 236, respectively. Daily ET_0 values ranged between 3.6 mm and 7.8 mm (Fig. 3a). Maximum air temperatures exceeded 32 °C on 27 of the 45 days, while minimum air temperatures varied between 14 and 26 °C (Fig. 3b). Daily net solar radiation values ranged between 2.4 MJ m⁻² and 12.1 MJ m⁻² during the experimental days and reached a maximum value of 12.1 MJ m⁻² on DOY 216 (Fig. 3c). Irradiance, air temperature, vapor pressure deficit and environmental demand were typical of Mediterranean climates (Saeger et al., 2019).

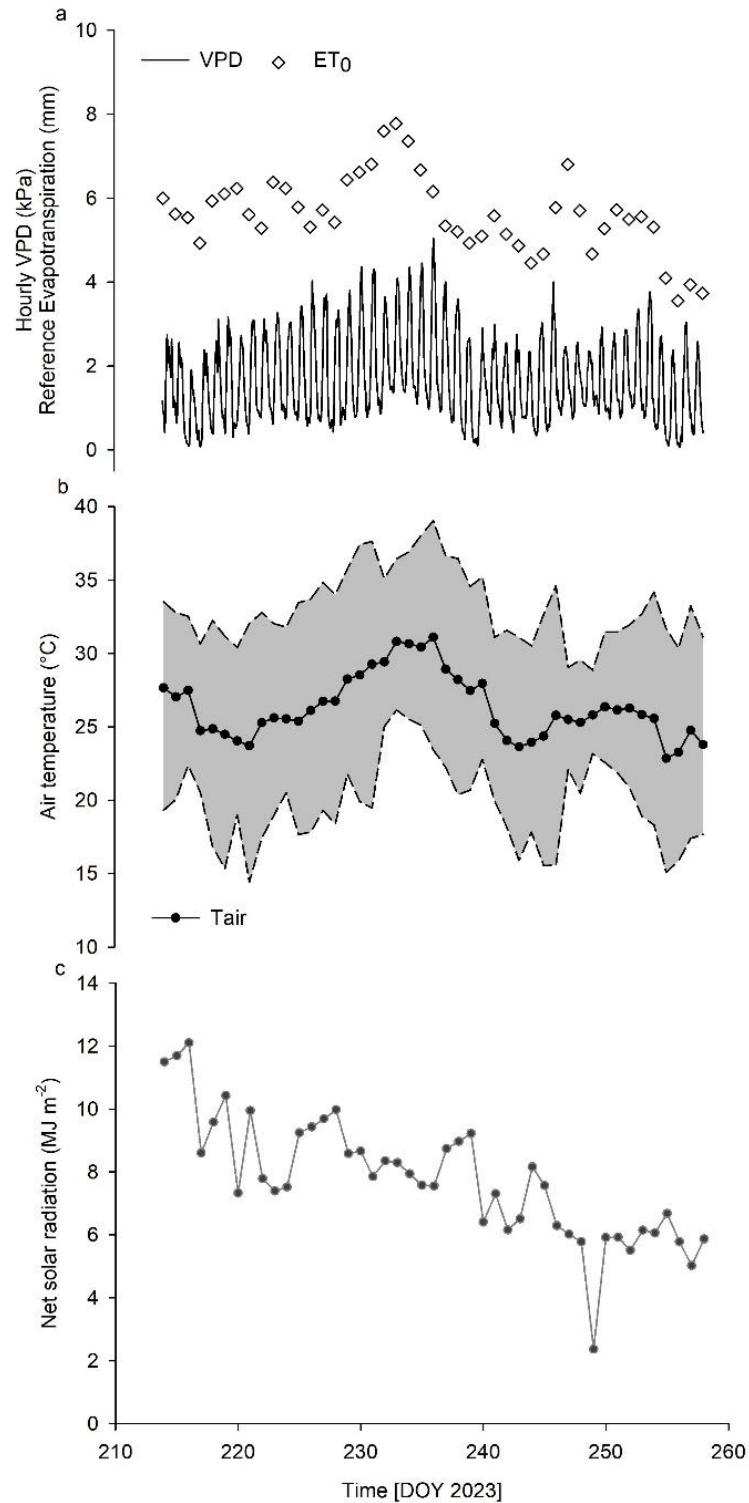


Fig. 3. (a) Hourly vapor pressure deficit (VPD) and daily reference evapotranspiration (ET_0), (b) daily air temperature (maximum, minimum and mean), (c) average daily net solar radiation during the irrigation experiment in a Mediterranean kiwifruit orchard. Time is expressed in day of the year [DOY].

Calculated and continuously measured soil water potential (ψ_{soil}) and sap flow (SF) from the well-watered (Fig. 4a) and drought (Fig. 4b) irrigation treatments were used as inputs to the model. Sap flow reflected environmental and soil water content changes during the days of the experiment. Transpiration during the night hours occurred in both irrigation treatments on days when higher VPD values were observed during the night (Fig. 3a). In the drought-stressed kiwifruit vines, the measured sap flow decreased with declining soil water availability, as indicated by the decrease in soil water potential, which was less pronounced in the first drought phase (from DOY 220 to DOY 226) and more pronounced in the subsequent drought phase (from DOY 244 to DOY 255). Upon full re-watering (from DOY 237 to DOY 243), sap flow of drought-stressed kiwifruit vines showed a slight increase again (Fig. 4b).

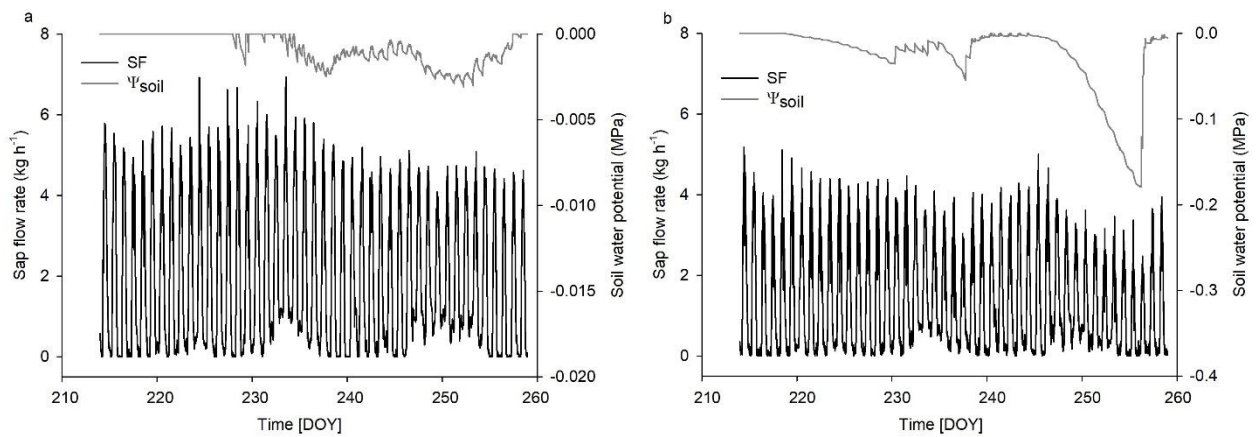


Fig. 4. Inputs used in the trunk water potential modelling process: sap flow rate (SF, black line) and soil water potential (ψ_{soil} , grey line) in the (a) well-watered and the (b) drought irrigation treatment. Time is expressed in day of the year [DOY].

The measured trunk water potential (ψ_{trunk}) of the well-watered vines showed values ranging from -0.02 MPa to -0.54 MPa, with daily oscillations and changes in the pre-dawn values, which can be partly attributed to the irrigation scheduling, in particular to changes in the irrigation volume and the splitting of the daily irrigation volume. The progressive drought stress developed in the drought irrigation treatment led to a decrease in ψ_{trunk} , reaching minimum values around -1.1 MPa in both drought phases, but with a different evolution of the stress within the plant, as shown by the differential response of ψ_{trunk} . Mechanistic modelling, which simulated stem hydraulics, provided ψ_{trunk} variations that matched closely with those measured in both well-watered and drought irrigation treatments (Fig. 5). The kiwifruit ψ_{trunk} model was calibrated using ψ_{soil} and SF as input variables and hydraulic capacitance (C) and resistance (R) as the calibrated parameters (Fig. 5a, b), or with C, R and the initial amount of water stored in the stem compartment (W) as calibrated

parameters (Fig. 5c, d). The simulated ψ_{trunk} varied according to the different types of calibration in both irrigation treatments. In the case of the well-watered treatment, a good fit between the simulated and measured ψ_{trunk} was observed in both calibrations based on only two (C and R) (Fig. 5a) or all three parameters (C, R and initial W) (Fig. 5c). When the initial value of W was included as a calibration variable, the simulations compared with the measured data showed that ψ_{trunk} became more responsive, with peaks associated with the daily recalibration of the amount of water present in the stem storage tissue, as illustrated in the detailed panels of Fig. 5 (I-II). While the simulation results of the two types of model calibration were both good for the well-watered treatment, more pronounced differences between the simulation results were observed for the drought treatment. In particular, the model calibration performed with only two parameters (C and R) was not sufficient to achieve a good comparison between the simulated and measured ψ_{trunk} during the second, more severe drought phase (from DOY 244 to DOY 255) (Fig. 5b). A distinct improvement in simulating the decreasing trend in ψ_{trunk} of the drought-stressed kiwifruit vines was achieved by including the initial value of W as a parameter in the calibration process. This resulted in a better fit between the simulated and measured ψ_{trunk} also during the second drought phase (Fig. 5d), compared to the model calibration with only C and R, which failed to describe the vine responses to severe drought, as detailed in the panel of Fig. 5 (III).

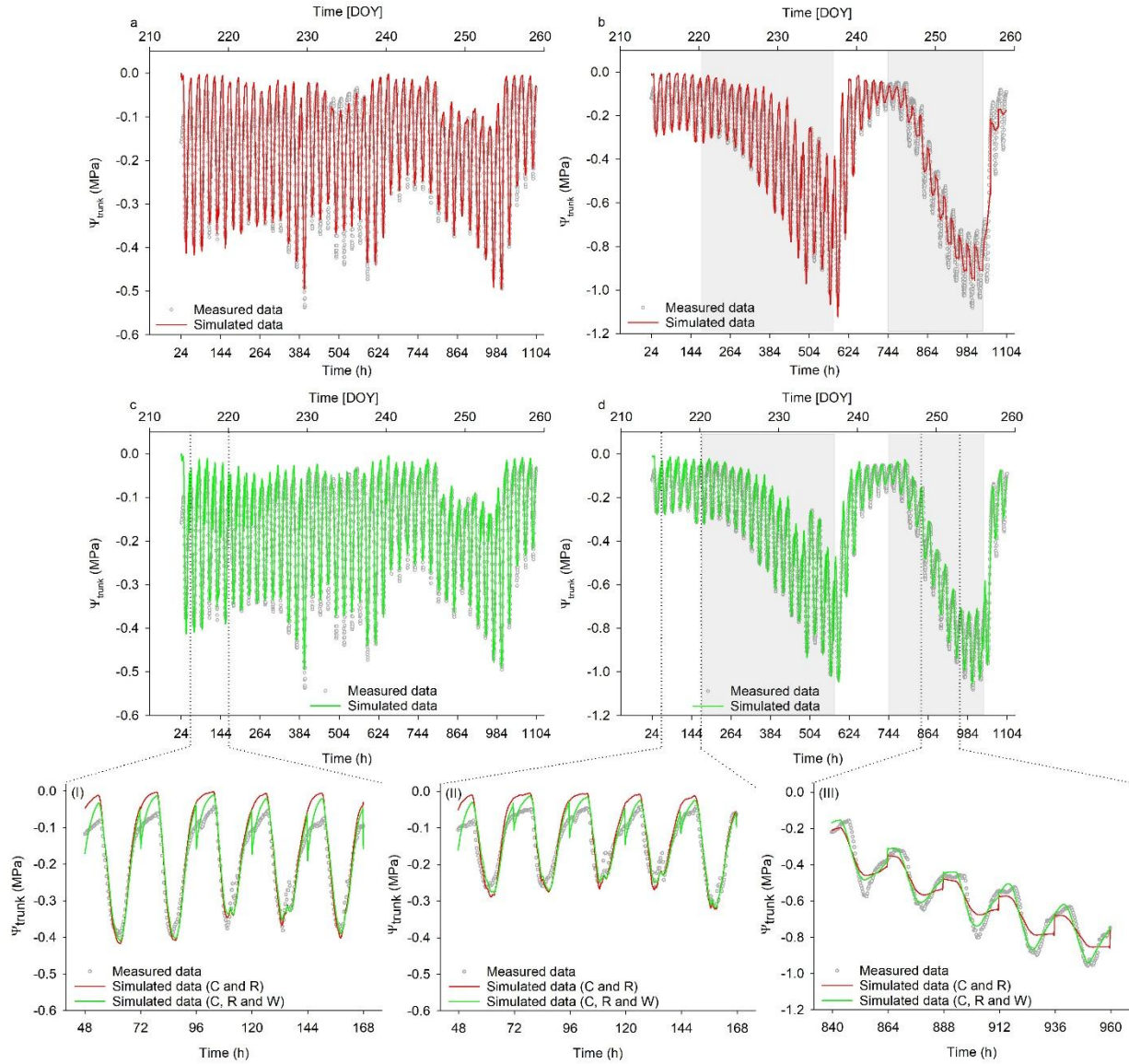


Fig. 5. Comparison between measured (dots) and simulated (lines) ψ_{trunk} using two calibration methods: i) hydraulic capacitance of the water storage tissue in the stem compartment (C) and xylem hydraulic resistance in the soil-to-stem segment (R) as calibrated parameters and ii) C, R and initial amount of water stored in the stem compartment (W) as calibrated parameters in the (a, c) well-watered and (b, d) drought irrigation treatments. Panels (I-III) illustrate specific differences between the model outputs obtained with the two types of calibration. The grey bands indicate the periods of reduced irrigation that led to the development of drought stress.

3.3.2 Variability of C and R in response to ψ_{trunk}

Clear linear relationships were found between the measured daily average ψ_{trunk} and the C and R values obtained from the model calibration performed with all three parameters (C, R and initial W), which were recalibrated daily based on a 1-day moving window (Fig. 6). In particular, C and ψ_{trunk} were linearly positively correlated, suggesting that improved plant water status is associated

with an increase in stem storage tissue capacitance, whereas R and ψ_{trunk} were linearly negatively correlated, suggesting that reduced plant water potential implies an increase in the hydraulic resistance. A weaker relationship was found for C by combining data from both the drought and well-watered irrigation treatments. Grey data points above the linear regression indicate a greater increase in the hydraulic capacitance of the stem water storage tissue due to re-watering of the drought stress treatment, which was carried out between DOY 237 and DOY 243. For C , these data points were excluded from regression calculation due to a different underlying process. The re-watering phase in the drought treatment allowed the vines to recover their ψ_{trunk} , which returned to the range of control values, and to increase their capacitance (Fig. 6a). Instead, a stronger relationship was found for R , combining data from both the drought and well-watered irrigation treatments (Fig. 6b). However, clear relationships were also maintained in the well-watered treatment for both C and R parameters, as shown in the detailed panels of Fig. 6 (I-II), indicating that changes in hydraulic capacitance and resistance also occurred within a narrow range of changes in ψ_{trunk} .

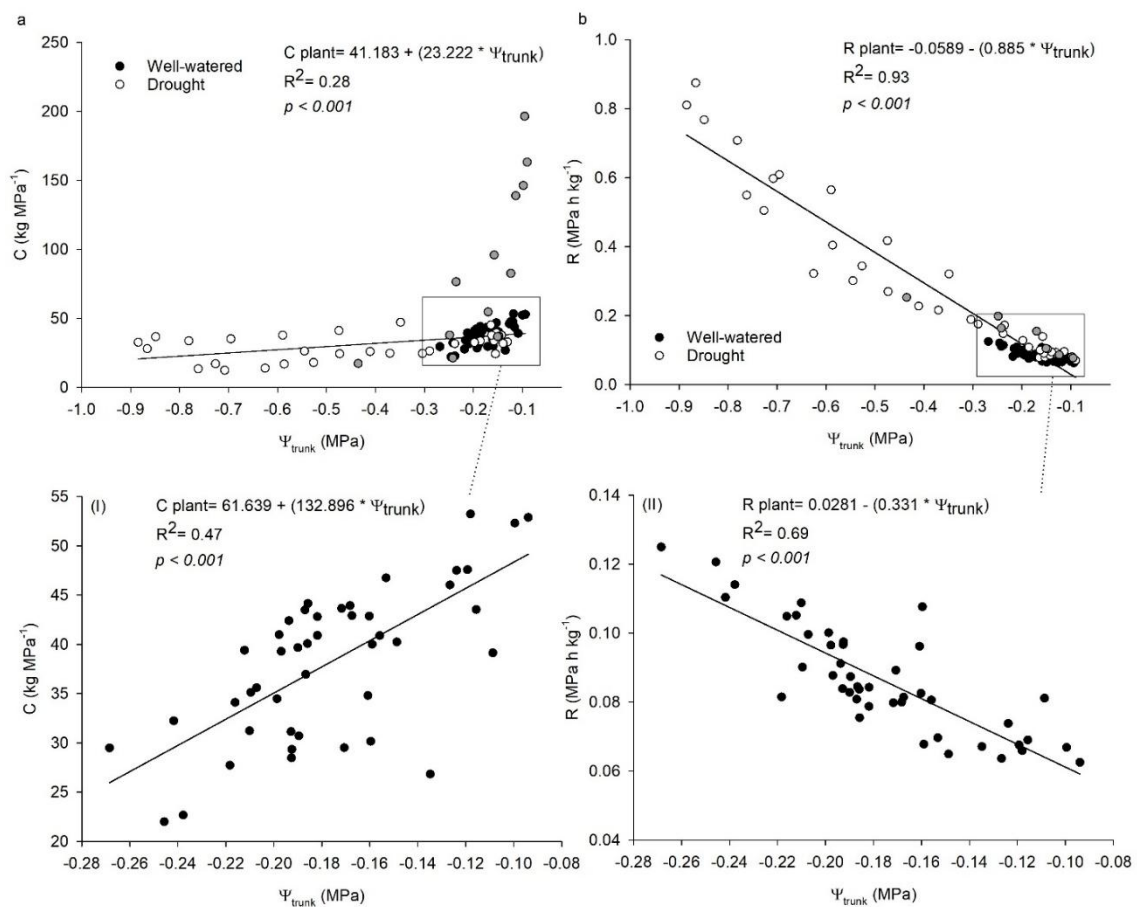


Fig. 6. Daily values of (a) hydraulic capacitance of the water storage tissue in the stem compartment (C) and (b) xylem hydraulic resistance in the soil-to-stem segment (R) as a function of daily average trunk water potential (ψ_{trunk}) in both well-watered and drought irrigation

treatments. Model calibration was performed with all three parameters (C, R and initial amount of water stored in the stem compartment, W). Grey points indicate the values of the drought treatment as affected by re-watering. Panels I-II illustrate the relationship of C and R with daily average ψ_{trunk} in the well-watered irrigation treatment.

Daily model calibration, which was performed using ψ_{trunk} measurements and a 1-day moving window, showed variation in both the hydraulic capacitance and resistance values (Fig. 7a, b). This adjustment of the parameter values was necessary to ensure a good model fit on all days (Fig. 5). The lowering of ψ_{trunk} in the drought-stressed vines during the drought periods resulted in lower C (Fig. 7a) and higher R values (Fig. 7b) compared to the well-watered vines. The hydraulic capacitance of drought-stressed vines showed lower values during the first drought period, a steeper increase in values due to full re-watering (i.e., from DOY 237 to DOY 243), before decreasing again, but to values that remained in the range of those of well-watered vines (Fig. 7a). On the other hand, the hydraulic resistance increased progressively during the first drought period, suggesting a progressive development of drought stress within the plant, and showed a steeper increase during the second drought period, peaking at values of $0.87 \text{ MPa h kg}^{-1}$, suggesting a more rapid and severe establishment of stress (Fig. 7b). Although the daily R values remained in a small range from 0.06 to $0.12 \text{ MPa h kg}^{-1}$ under adequate soil water availability, a daily variability in hydraulic resistance was observed (Fig. 7b), highlighting the responsiveness of the parameter, which can contribute to the understanding of plant behavior, according to the relationship found with ψ_{trunk} in the well-watered treatment (Fig. 6, panel II).

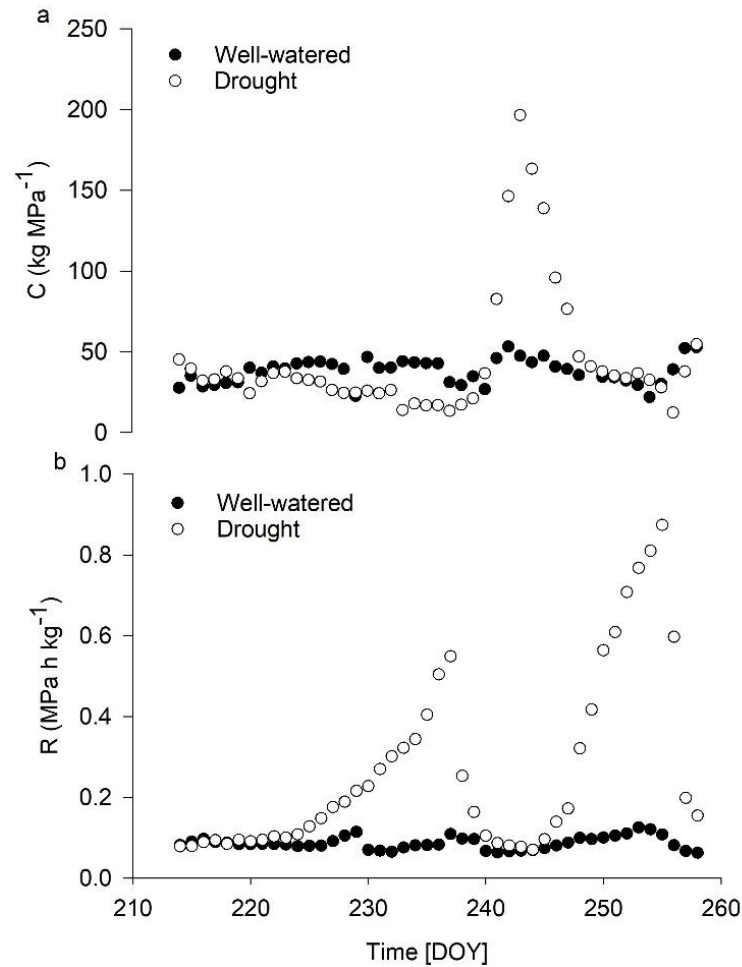


Fig. 7. Time evolution of the daily calibrated model parameter values during the irrigation experiment in both well-watered and drought-stressed kiwifruit vines: (a) hydraulic capacitance of the water storage tissue in the stem compartment (C); (b) xylem hydraulic resistance in the soil-to-stem segment (R). Time is expressed in day of the year [DOY].

3.3.3 Comparison of calibrated model parameters

The calibrated model parameters (C and R) did not vary between the two types of model calibration (i.e. based on only two or three parameters by including the initial value of W in the calibration process) in the well-watered dataset (Fig. 8a, b). On the other hand, a different dynamic of C and R parameters was observed when comparing model calibrations under more severe drought conditions. In this case, the model calibration performed with only two parameters was not able to provide reliable calibrated values of C and R (see peaks, Fig. 8c, d). Differences in the calibrated model parameters between the two types of model calibration explained the failure of the two-parameter based model calibration to simulate ψ_{trunk} when severe drought occurred, and supported the improvement in the calibrated values (C and R) when the initial value of W was included in the calibration process, which also allowed a better simulation of ψ_{trunk} (Fig. 5b, d).

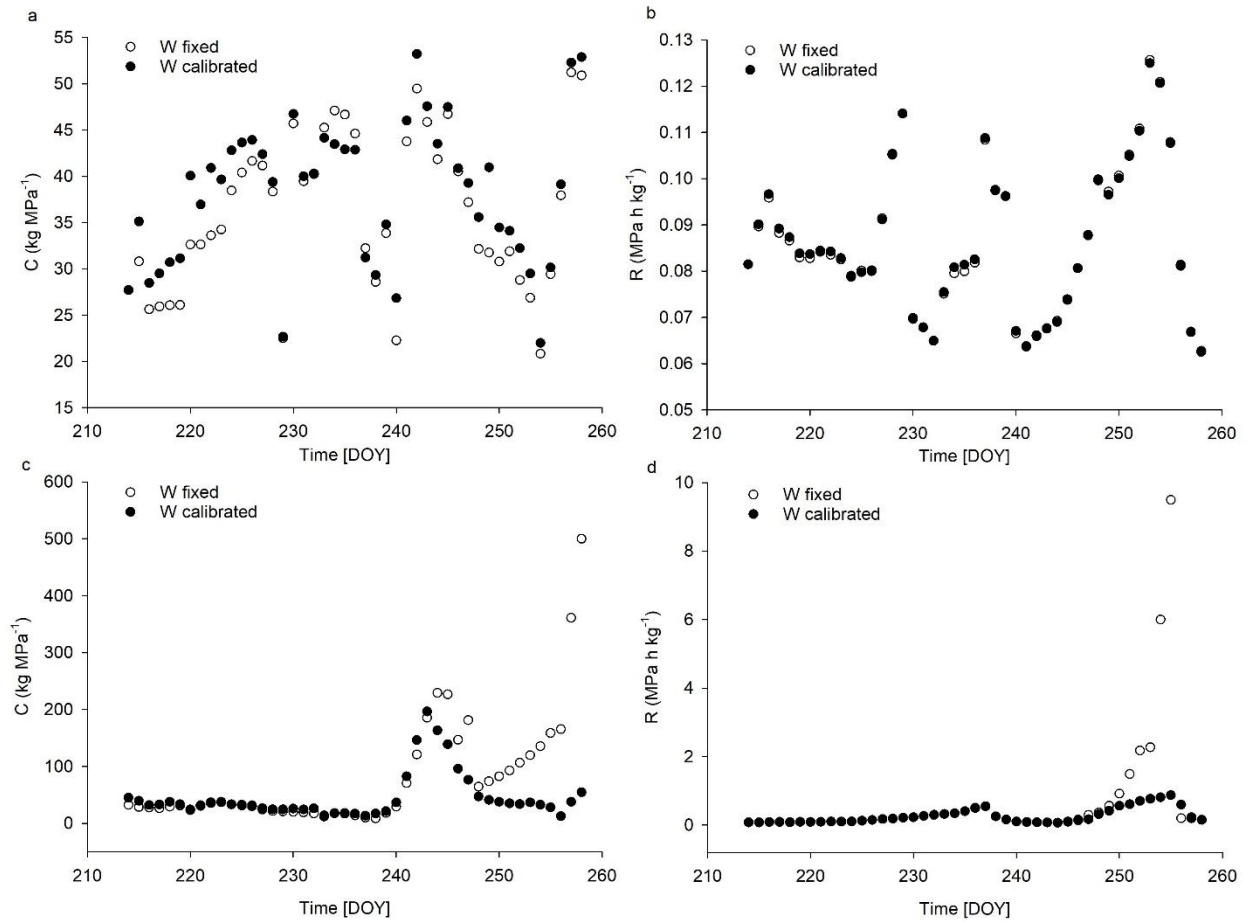


Fig. 8. Comparison of daily calibrated model parameter values (hydraulic capacitance of the water storage tissue in the stem compartment, C , and xylem hydraulic resistance in the soil-to-stem segment, R) obtained from model calibration with adaptation of two (C and R) or three (C , R and initial amount of water stored in the stem compartment, W) parameters during the irrigation experiment in (a, b) well-watered and (c, d) drought irrigation treatments. Time is expressed in day of the year [DOY].

The two model calibration approaches differed in that one kept the initial value of W fixed at the value obtained from the end of the previous 1-day moving window, while the other included the initial value of W as a calibration parameter that was recalibrated daily together with C and R . Again, the comparison of the initial W values between these model calibration approaches showed no relevant differences for the well-watered dataset (Fig. 9a). However, during severe drought, the two-parameter model calibration with a fixed initial W value failed, as shown by negative initial W values (Fig. 9b).

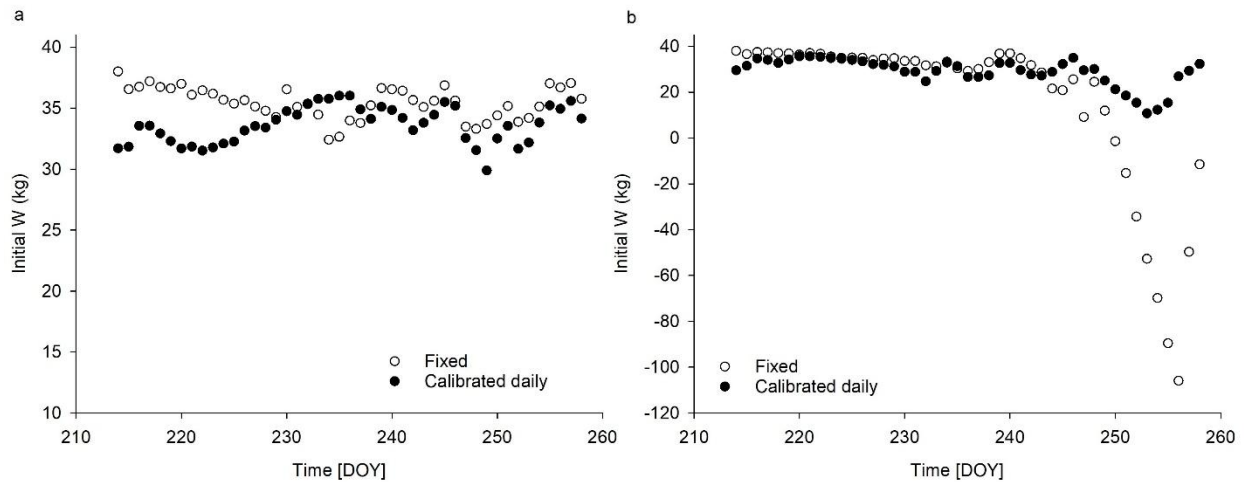


Fig. 9. Comparison of initial values of stored water (W) using two model calibration approaches: one where the initial value of W was fixed at the value obtained from the end of the previous 1-day moving window and only two parameters (C and R) were calibrated, and another where the initial value of W , alongside with C and R , was recalibrated daily in the (a) well-watered and the (b) drought irrigation treatment. Time is expressed in day of the year [DOY].

3.4 Discussion

3.4.1 Practical insights into model calibration

An existing water flow and storage model (Steppe et al., 2006; 2008), originally designed to simulate stem water potential among other variables, was adapted for use in this study to simulate trunk water potential and estimate hydraulic parameters associated with water flow in kiwifruit under drought stress. The model uses continuously measured sap flow and soil water potential data as inputs, while trunk water potential measurements are used for calibration. As the trunk water potential was measured continuously, it was possible to derive the hydraulic capacitance and resistance of kiwifruit vines on a daily basis under well-watered and progressive drought conditions, which helps to understand the physiology of kiwifruit vines and to determine the trends and order of magnitude of key plant hydraulic parameters. The adapted model was able to simulate the measured data (Fig. 5). Distinct improvements in the simulation of trunk water potential changes under drought stress were obtained by accurately choosing the size of the moving window, which is an important issue to consider when performing model calibration (Steppe et al., 2008), and the parameters to be considered for calibration purposes. In particular, for a successful application of the mechanistic model in the present study, a moving window of small size (i.e., 1 day) was chosen. This window size allowed the day-to-day variability of model parameters to be detected under both well-watered and soil drying conditions, thus providing more accurate

parameter estimates and model simulations compared to a larger window size (Steppe et al., 2008). A sensitivity analysis of the model revealed the identifiable parameters (according to De Pauw et al., 2008a, b) that can be unambiguously estimated during model calibration from the available measurements: the hydraulic capacitance of the water storage tissue in the stem compartment, the xylem hydraulic resistance in the soil-to-stem segment, and the initial amount of water stored in the stem compartment. Based on the results of the identifiability analysis, a first attempt was made to calibrate two identifiable parameters (C and R) on both well-watered and drought datasets. The model showed a good fit to the measured trunk water potential data in the well-watered dataset (Fig. 5a), but was unable to adequately describe the drought dataset, particularly in the second drought phase (Fig. 5b). When three identifiable parameters were calibrated on both well-watered and drought datasets, the model was able to accurately describe the steeper decline in trunk water potential (Fig. 5d) due to more severe soil drying (Fig. 4b). The inclusion of the third identifiable parameter in the calibration process had negligible effects on the calibrated parameters (C and R) for the well-watered (Fig. 8a, b) and much of the drought (Fig. 8c, d) datasets. However, during the severe drought phase, these differences became evident, with the calibration of the initial value of stored water also improving the other calibrated variables. The inclusion of initial stored water (W) in the calibration process allowed this parameter to be adapted daily during the moving window calibration, rather than relying on a fixed initial value and subsequent calculated state values of W at the start of each window (Fig. 9). Although a fixed initial value was adequate to allow the model to describe the behavior of kiwifruit vines under well-watered conditions (Fig. 9a), the initial stored water reached unrealistic values in the second, more severe drought phase (Fig. 9b). This suggests that the initial value of stored water should be included as a calibration variable when there is a rapid drop in trunk water potential (i.e., during severe drought stress). The results of this study clearly show the importance and advantages of performing an identifiability analysis, which improves the model to better describe the experimental data, especially under drought stress conditions, according to De Pauw et al. (2008a). Indeed, in the present study it was advisable to include the initial amount of water stored in the stem compartment as a calibration parameter, especially during the more severe drought stress, as it improved the simulation of trunk water potential and enabled improved estimates of key hydraulic parameters to describe the response of kiwifruit vines to drought. Contrarily, a variable initial W value may not improve model performance under well-watered conditions as C and R are expected to remain almost constant. The adapted model for continuous modelling of kiwifruit vine water status and hydraulic behavior was then successfully applied under conditions ranging from wet to more or less severe drought.

3.4.2 Modelling vine drought response

The study of plant hydraulics is of increasing interest to better understand the impacts of drought and to predict how plants will respond to climate change (Sperry and Love, 2015; De Swaef et al., 2022; Torres-Ruiz et al., 2024). Mechanistic plant models, in combination with plant measurements, represent promising tools to increase knowledge on various plant physiological processes and hydraulic functioning (Steppe et al., 2015b; De Swaef et al., 2022), showing great potential for understanding the effects of limiting soil water availability and the hydraulic response of plants to drought (Baert et al., 2015; Salomón et al., 2017). The nature of hydraulic constraints on plant functioning is increasingly being addressed (Williams et al., 2001), and several variants of plant hydraulic models have been proposed over the last two decades (Paschalis et al., 2024). In particular, the study of tissue hydraulic capacitance and storage capacity and their inclusion in models has received increasing attention due to the dynamic nature of water transport throughout the plant (Steppe et al., 2015b). A key parameter widely included in most models is hydraulic resistance in the soil-to-plant segment, as it greatly influences plant water status and responses to drought (Baert et al., 2015). Therefore, one of the objectives of our study was to use an effective model to derive key hydraulic parameters, such as hydraulic capacitance and resistance, that are otherwise difficult to measure and that can help characterize the drought responses and mechanisms of kiwifruit vines. Our model was adapted from Steppe et al. (2006); (2008) and simplified to describe only the processes occurring in the stem compartment, with a water flow resistance (R) and accounting for water storage in the stem by including plant hydraulic capacitance (C) (Fig. 2). Although most models do not account for drought-induced C variability, the dynamic nature of C should be considered in mechanistic water transport models to better understand the hydraulic functioning of plants (Salomón et al., 2017). Woody plants absorb water, store it at night in several compartments and release it during the day for leaf transpiration, representing an important mechanism to regulate the dynamics of transpiration (Gao et al., 2021). Hydraulic capacitance reflects the ability of a tissue to store and release water into the xylem in response to a given change in tissue water potential (Steppe et al., 2006; Sholz et al., 2007, 2011; Meinzer et al., 2010). Capacitive water release into the transpiration stream can buffer daily fluctuations in xylem tension by reducing or slowing down transpiration-induced xylem pressure drops (Meinzer et al., 2009; McCulloh et al., 2014; Epila et al., 2017; Steppe, 2018), thereby reducing the risk of xylem embolism and hydraulic failure under dynamic conditions (Scholz et al., 2011). Instead, R represents the resistance to water flow and has been shown to vary between species, in part due to differences in xylem vessels structure and size (Tyree and Ewers, 1991;

Sperry et al., 2006), and the model-derived parameter has previously been linked to a realistic wood anatomical basis (Steppe and Lemeur, 2007).

The hydraulic parameters of yellow-fleshed kiwifruit vines under Mediterranean field conditions have been poorly studied, and this is the first study to use a mechanistic plant water flow model to unravel kiwifruit drought behavior. Model calibrations and outputs from this study revealed that hydraulic parameters such as C and R fell within realistic ranges and highlighted important changes in the hydraulic behavior of well-watered and drought-stressed kiwifruit vines (Fig. 6). In particular, the C values obtained in our study ranged from 22 to 53 kg MPa⁻¹ for the well-watered vines, while in the drought-stressed vines C progressively decreased during the drought periods, reaching lower values of about 12 kg MPa⁻¹ (Fig. 7a). Instead, the R values ranged from 0.062 to 0.12 MPa h kg⁻¹ for the well-watered vines, while in the drought-stressed vines R progressively increased during the drought periods, reaching higher values of about 0.87 MPa h kg⁻¹ (Fig. 7b). Data on C are now available for several species from a variety of ecosystems, with different architectures (stem diameter and tree size), wood types and densities, but comparison with values in the literature is not straightforward due to inconsistent use of capacitance units (Scholz et al., 2011). A wide range of published values of R can also be found in different units (Verbeeck et al., 2007). Calibrated values for C were found to range from 23.7 to 69.0 kg MPa⁻¹ for Scots pine (Verbeeck et al., 2007), and approximately 212 and 940 mg MPa⁻¹ for beech and oak trees, respectively (Steppe and Lemeur, 2007). Calibrated values for R were found to range from 0.46 to 1 MPa h kg⁻¹ for Scots pine (Verbeeck et al., 2007), and approximately 0.18 and 0.11 MPa s mg⁻¹ for beech and oak trees, respectively (Steppe and Lemeur, 2007).

The linear relationships between ψ_{trunk} and C and R strongly confirm that variations in plant hydraulic properties are strictly related to variations in plant water relations and status (Torres-Ruiz et al., 2024). In particular, C was found to decrease with more negative ψ_{trunk} values (Fig. 6a), whereas R showed an increase (Fig. 6b). These results align with previous studies on various woody species that have examined changes in C and R across a gradient of plant water potential. These studies consistently report that C decreases and R increases with more negative plant water potential values, supported by strong correlations (Meinzer et al., 2003, 2009; Sholz et al., 2007; Salomón et al., 2017). A previous study on pot-grown kiwifruit vines (cv. ‘Hayward’) found that the water released to the transpiration stream from different tissues (leaves, shoots, branches, roots and fruits) corresponded to 8.5% of total transpiration (measured from pre-dawn to 2 p.m.) for control vines, 9.6% for moderately stressed vines and 5.1% for vines with the least water available (Nuzzo et al., 1997). These values are relatively low compared to other fruit trees, such as olive trees, whose leaves can use about 60% of their water reserves for transpiration without irreversible

damage (Sofa et al., 2008). In addition, the high root/canopy ratio and the efficiency of the vascular system allow kiwifruit vines to satisfy approximately 91% of their transpiration demand directly through root water uptake from the soil, under non-limiting soil water availability conditions (Nuzzo et al., 1997). The model-derived capacitance values, which showed a progressive decrease with increasing drought stress, are consistent with the observed tendency of kiwifruit vines to reduce the release of stored water into the transpiration stream under drought stress. Instead, hydraulic resistance was found to significantly increase under persistent drought conditions in woody trees and grapevines (Baert et al., 2015; Peters et al., 2021). As soil water content decreases, hydraulic resistance to water flow in the soil increases and soil matric potential decreases, resulting in steeper water potential gradients within the plant (i.e. more negative plant water potential values) (Scharwies and Dinneny, 2019). Large xylem vessels, reported to be between 0.12 and 0.5 mm in both root and stem of kiwifruit, provide a very low resistance pathway for water movement within the vine (McAneney and Judd, 1983), but at the same time could contribute to a more frequent occurrence of embolism formation, even at relatively high xylem water potential values (Clearwater and Clark, 2003). The increase in R , which has been found to increase as the soil dries (McAneney and Judd, 1983), may also be driven by physical changes in the xylem, such as embolism formation resulting from drought stress. The C and R parameters derived through model simulation and calibration allowed further characterization of the hydraulic behavior of yellow-fleshed kiwifruit vines under different irrigation conditions. This understanding is particularly valuable in semi-arid Mediterranean environments, where optimized water management is increasingly critical.

3.4.3 Potential use of mechanistic models for irrigation

In addition to advancing our understanding of the hydraulic functioning of kiwifruit vines and the dynamics of water transport, the modelling process can be integrated into a practical automated monitoring system. This approach enables the development of a tool for real-world irrigation applications, as previously demonstrated by Steppe et al. (2008). Plant measurements, based on sap flow and trunk water potential, are fed into a mechanistic plant model, to continuously derive key hydraulic parameters that can be used as promising indicators to detect drought stress conditions and thus guide irrigation decisions. Our drought stress experiment shows that measured ψ_{trunk} deviated from the expected pattern (well-watered vines) once drought stress was imposed by modulating irrigation (Fig. 5). Integrating these measurements with sap flow data and mechanistic plant modelling enables the daily derivation of plant hydraulic parameters that are otherwise challenging to measure. In addition, as the measurements from the plant sensors are continuous,

the hydraulic functioning of vines or fruit trees can be modelled continuously, rather than being limited to discrete observations, contributing to the study of the hydraulic functioning of plants under natural conditions throughout the growing season. These hydraulic parameters have been shown to strongly correlate with plant water status, providing a unique and valuable perspective on plant behavior under varying water conditions. However, the relationship between C and R with ψ_{trunk} was also observed under well-watered conditions, demonstrating that the calibrated parameters changed within a narrow range of plant water potential (e.g., ψ_{trunk} ranged from approximately -0.1 MPa to -0.3 MPa under well-watered conditions). This highlights their sensitivity and underscores their strong correlation with the water status of the vines (Fig. 6, panels I-II). The investigated variations and dynamics of C and R of yellow-fleshed kiwifruit vines in relation to plant water status highlight the potential of these parameters to be used as indicators of drought stress and for irrigation purposes, the opportunities of which should be further explored. The modelling approach developed can be effectively tested and transferred to various fruit crops, providing the basis for future research to implement practical tools that can be used in real-world irrigation applications.

3.5 Conclusions

As drought events are expected to increase in the coming years, especially in the Mediterranean area, it is important to gain knowledge on the drought response and hydraulic behavior of crops under field conditions. This study combines plant sensor measurements with a mechanistic water transport model to enable real-time, *in-situ* field monitoring of hydraulic capacitance and resistance, revealing the drought response of kiwifruit vines. In particular, the kiwifruit trunk water potential model showed a progressive decrease in the hydraulic capacitance and a substantial increase in the hydraulic resistance with increasing soil and plant water deficit. To date, several models have been developed to gain a better understanding of the plant processes related to drought stress. However, since sensors are still not cost-effective and taking measurements at farm level is challenging, modelling approaches have tended to remain within the scientific and research communities rather than being used by farmers. We strongly believe that sensor technologies will be increasingly adopted over the next few years, leading to a new generation of irrigation decision tools that use plant monitoring and mathematical plant modelling to inform irrigation. Within this framework, the used model not only introduces relevant information on the hydraulic functioning of kiwifruit vines in response to drought stress in a Mediterranean environment, but also shows great potential as a basis for developing real-time, plant-based irrigation management that can be transferred to various fruit crops.

3.6 References

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4. Assessment of drought stress sensitivity of kiwifruit rootstocks and grafting combinations through integrated physiological and phenotyping analysis

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Abstract

Kiwifruit is known to have high water requirements and is sensitive to reduced water availability. Many of the major kiwifruit growing areas are expected to face increasing water scarcity due to climate change. Despite the demonstrated impacts of drought stress on kiwifruit, the sensitivity of different kiwifruit genotypes to drought remains poorly described. The present study aimed to evaluate the physiological and phenotypic drought responses of three kiwifruit rootstocks ('Bounty 71', *Actinidia macrosperma*; 'D1' and 'Hayward', *Actinidia chinensis* var. *deliciosa*) and scion/rootstock combinations, in which a yellow-fleshed kiwifruit cultivar ('Zesy002', *A. chinensis* var. *chinensis*) was grafted on each rootstock.

A pot experiment was conducted at the ALSIA 'Phenolab', part of the Phen-Italy infrastructures, using non-destructive RGB and NIR imaging combined with leaf gas exchange measurements. Potted vines were subjected to continuous well-watered conditions or to a mild drought stress cycle, imposed by progressively reducing pot water availability (up to about 35% field capacity), followed by 5 days of full re-watering.

Based on physiological measurements, Hayward and D1 rootstocks were more affected by drought than Bounty 71, with photosynthetic rate reduced due to lower stomatal conductance under limited pot water availability. Drought-induced physiological (reduced stomatal conductance and photosynthetic rate, increased intrinsic water use efficiency, and decreased instantaneous carboxylation efficiency) and phenotypic (changes in projected shoot area and leaf color) responses differed among combinations, with Hayward identified as the most drought-sensitive. Complete recovery of both physiological and phenotypic parameters was observed in all combinations, suggesting the ability to cope with short-term and mild drought stress.

The results support the use of phenotypic tools combined with leaf gas exchange measurements for a more comprehensive screening of drought-tolerant genotypes in kiwifruit.

4.1 Introduction

In recent years, kiwifruit has become an important fruit crop with a growing global distribution, attracting the interest of several countries in both the northern and southern hemispheres (Ferguson et al., 2023). The kiwifruit industry in the last decade has been characterized by the increasing importance of China, which produces more than half of the world's kiwifruit, followed by Italy in the northern hemisphere, while New Zealand and Chile are the most important countries in the southern hemisphere (Costa et al., 2018). The availability of irrigation has made it possible to increase the total area of suitable land by meeting the deficit in areas where rainfall is insufficient to match the evaporative demand (Salinger and Kenny, 1995), to successfully grow kiwifruit in Mediterranean countries (Chartzoulakis et al., 1993), and to expand its cultivation in areas with different climates. The cultivation of kiwifruit in Italy extends over the whole length of the country, with the most varied environmental conditions (Costa et al., 2018).

Climate variability and the occurrence of extreme weather events will continue to increase in the coming years, posing a global risk to kiwifruit production (Cradock-Henry, 2017; Chamberlain, 2023). Kiwifruit producing regions are vulnerable to changing environmental conditions (Rajan et al., 2024) and exposed to increasing water scarcity due to climate change (Calderón-Orellana et al., 2021), especially in the Mediterranean basin, which will be affected by increases in temperature and evaporative demand and decreases in precipitation (Lionello and Scarascia, 2018; Prada et al., 2024).

Drought stress is one of the major environmental stresses to plants, negatively affecting plant development and performance and causing changes at the physiological, morphological, biochemical and molecular levels (Salehi-Lisar and Bakhshayeshan-Agdam, 2016; Seleiman et al., 2021; Sato et al., 2024). Kiwifruit has high irrigation requirements, especially when grown in Mediterranean environments (Chartzoulakis et al., 1993; Holzapfel et al., 2000; Calderón-Orellana et al., 2021), where it can easily experience water deficits due to atmospheric conditions that typically become critical during the summer, combined with inadequate water supply (Chartzoulakis et al., 1993) and irrigation restrictions. Water deficit in kiwifruit has been found to reduce fruit expansion and growth (Judd et al., 1989); decrease photosynthetic activity, leaf area development, and photosynthate partitioning, which in turn affects vine growth (Chartzoulakis et al., 1993; Liang et al., 2019); increase leaf and cell membrane damage and oxidation (Wang et al., 2011; Bao et al., 2020); and increase fruit sunburn damage (Calderón-Orellana et al., 2021). Photosynthesis, an important plant process, is very sensitive to drought stress (Sharma et al., 2020). Water deficit imbalances the plant's water status and induces the primary response of all plants to drought stress, represented by a reduction in transpiration through stomatal closure (Sharma et al.,

2020; Ahluwalia et al., 2021; Seleiman et al., 2021). Stomatal closure limits water loss, but it also reduces CO₂ assimilation, which in turn affects photosynthesis (Ahluwalia et al., 2021; Baldi et al., 2024; Mansoor and Chung, 2024; Qiao et al., 2024). Montanaro et al. (2007) reported that kiwifruit is able to cope with the Mediterranean environment through low stomatal conductance and enhanced non-photochemical quenching, sacrificing carbon gain for water conservation, which was also actuated under severe drought stress.

Although the drought stress response of kiwifruit vines has been extensively studied, comparisons between rootstocks and information on rootstock-induced drought tolerance in kiwifruit scion cultivars are still lacking. The relatively recent domestication of kiwifruit has involved little breeding research and rootstock selection compared to other temperate tree and vine crops. However, much material is available for the selection of new rootstocks from the rich genetic diversity of *Actinidia* species (Thorp and Barnett, 2023).

Given the impacts of climate change and the spread of kiwifruit to semi-arid areas, it is becoming increasingly important to evaluate the drought response of different kiwifruit genotypes to select and identify more resilient rootstocks and cultivars. A previous study of the drought response of different kiwifruit rootstocks showed that moderate drought impaired photosynthesis, increased cell membrane damage and reactive oxygen species-associated enzyme activity differently among genotypes. However, as drought intensified to severe levels, the effects on kiwifruit growth, photosynthesis, and oxidative damage worsened in all genotypes (Bao et al., 2024). Recent studies have shown that rootstocks belonging to *A. macrosperma* are likely to be tolerant to drought (Zhong et al., 2018) and intermittent waterlogging (Kataoka et al., 2021; Beppu et al., 2022), proving to be more resilient to adverse soil and environmental conditions.

To assess plant growth, physiological, molecular, and biochemical responses, the development of new tools to monitor plant responses to drought, such as imaging technologies, will help to understand plant performance in different environments and bridge the phenotype-genotype gap (Chaves et al., 2003). Automated platforms can improve the throughput of plant phenotyping by using integrated sensors, such as visible light, near-infrared, fluorescence, multispectral, and hyperspectral (Fu and Jiang, 2022), allowing for the collection of large amounts of high-quality data in a continuous, non-destructive, and non-invasive manner (Danzi et al., 2019).

Plant phenotyping can effectively detect and monitor changes induced by drought stress, thus contributing to the understanding of how plants cope with water scarcity (Mansoor and Chung, 2024). Morphological traits measured through phenotyping, such as plant height, leaf area, leaf greenness, leaf angle, and plant wilt degree, can be indicators to quantify plant stress responses (Li et al., 2021). Physiological indexes such as leaf water content, transpiration rate, and chlorophyll

fluorescence, are increasingly being used in the emerging physiology-based phenotyping of stress response (Li et al., 2021). Briglia et al. (2019; 2020) evaluated the drought response of grapevines using morphological traits such as leaf greenness, leaf area development, and changes in leaf angle. Previous studies have performed image-based assessment of drought response in tomato and rice (Janni et al., 2019; Kim et al., 2020) and evaluated the drought tolerance or susceptibility of different wheat genotypes (Danzi et al., 2019; Danzi et al., 2022). Morpho-physiological changes in kiwifruit rootstocks and grafting combinations under short-term waterlogging stress were assessed in a previous study, revealing that Bounty (*A. macrosperma*) was the more tolerant genotype compared to D1 and Hayward (*A. chinensis* var. *deliciosa*) (Calabritto et al., 2025). In the present study, an approach combining high-throughput plant phenotyping (HTPP) and leaf gas exchange measurements is used in a pot experiment to i) assess the tolerance/susceptibility of different kiwifruit rootstocks to drought stress and recovery and ii) evaluate the response of the yellow-fleshed kiwifruit scion grafted on different rootstocks to drought and recovery.

4.2 Materials and methods

4.2.1 Plant material and growth conditions

A pot experiment was carried out during the 2023 season at the ALSIA ‘Metapontum Agrobios’ Research Centre in Metaponto (southern Italy). The plant material consisted of 30 two-year-old rootstocks of ‘Hayward’ (H) (*A. chinensis* var. *deliciosa*), ‘Bounty 71’ (B) (*A. macrosperma*) and ‘D1’ (D) (*A. chinensis* var. *deliciosa*) and 42 one-year-old scions of cv. ‘Zesy002’ (*A. chinensis* var. *chinensis*) grafted on each rootstock (i. e., scion/rootstock: Z/H, Z/B and Z/D), resulting in 10 and 14 replicates per treatment, respectively. All kiwifruit vines were transplanted in March in blue pots of 19.5 cm height and 16 cm wide (3 L capacity) with the same amount of medium and grown in an unheated and unconditioned greenhouse under natural light conditions. The vines were randomly distributed to reduce any effect of greenhouse microclimatic variations. Environmental conditions in the greenhouse during the experiment were: minimum temperature of around 20 °C and maximum temperature of around 40 °C, with relative humidity ranging from a minimum of 30% to a maximum of 85%. The potting medium was a mixture of acid peat and pumice (pH 6, bulk density 0.23 g cm⁻³ and 90% porosity). All vines were trained with a single main shoot and pruned to a similar leaf area (approximately 10 leaves each). Each vine was tied to a wooden support, which was painted blue for image acquisition, segmentation and analysis. The pots were labelled with a barcode for automatic weighting, watering and identification of the vines during image acquisition on the plant phenomics platform.

4.2.2 Irrigation treatments and soil water status

From transplanting to the start of the experiment, all the vines received amounts of water consistent with field capacity (FC).

Prior to the start of the experiment, each pot was fully watered and the excess water was allowed to drain until no water came out of the bottom of the pots and stable pot weights were achieved. The average weights were used as pot weight at 100% pot water content, referred to as maximum pot weights.

From 3 July, the vines were assigned to two irrigation treatment groups (i.e., control, Ctrl and drought stress, S), following a complete randomization experimental design with five vines from each rootstock and seven vines from each scion/rootstock combination.

Soil water availability (SWA) was calculated as follows (Sapeta et al., 2013):

$$SWA = \left[\frac{(Pot\ Weight - Minimum\ Pot\ Weight)}{(Maximum\ Pot\ Weight - Minimum\ Pot\ Weight)} \right] * 100 \quad (1)$$

The minimum pot weight was considered to be the pot's weight containing a completely dry substrate. For this measurement, the soil was oven-dried until a constant weight was reached.

In the Ctrl treatment, vines were grown continuously under well-watered conditions. The soil water content of the pots was continuously maintained close to FC, with water losses replenished by automatic weighing and watering of the pots two/three times per day by the platform, thus restoring the maximum pot weight defined at FC.

Drought stress was imposed by gradually reducing pot soil water availability (by 20% to up to 35% based on pot weight), followed by five days of full re-watering (R).

4.2.3 Leaf gas exchange measurements

Stomatal conductance (gs, mol H₂O m⁻² s⁻¹), transpiration rate (E, mmol H₂O m⁻² s⁻¹), net photosynthetic rate (A, μmol CO₂ m⁻² s⁻¹), leaf temperature (T_{leaf}, °C), photosystem II operating efficiency (ΦPSII) in the light-adapted state and electron transport rate (ETR, μmol e⁻ m⁻² s⁻¹) were measured using a portable gas exchange system (Li-Cor 6800, Li-Cor Inc., Lincoln, NE, USA). Measurements were taken at different levels of SWA reduction (-17% FC, -22% FC, and -35% FC in rootstocks and 19% FC, 23% FC and -37% FC in scion/rootstock combinations) at the time of maximum efficiency (10:00-12:00 h, solar time). In addition, a diurnal trend of leaf gas exchange was measured at approximately three-hour intervals (8:00, 10:00, 13:00 and 16:00 h, solar time) when SWA reached maximum reduction. The same physiological parameters were measured during the hours of maximum efficiency (10:00-12:00 h, solar time) during the five-day recovery period. Intrinsic leaf water use efficiency (A/gs, μmol CO₂ mol⁻¹ H₂O) and instantaneous

carboxylation efficiency (A/C_i , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} \text{ ppm}^{-1}$) were also calculated. Leaf gas exchange measurements were performed at an ambient CO_2 concentration of $400 \mu\text{mol mol}^{-1}$, with temperature and irradiance maintained at the prevailing external environmental conditions according to Medrano et al. (2010), and an operating flow rate of $500 \mu\text{mol s}^{-1}$.

To reduce the effects of leaf position and developmental age, a fully expanded and sun-exposed leaf with no visual symptoms of stress was chosen from the mid-shoot region of four randomly selected vines for each irrigation treatment, rootstock and scion/rootstock combination.

4.2.4 High-throughput plant phenotyping

For HTPP measurements, all vines were non-destructively and non-invasively phenotyped using a Scanalyzer 3D plant phenotyping platform (LemnaTec GmbH, Aachen, Germany). The platform ‘PhenoLab’ is located at the ALSIA ‘Metapontum Agrobios’ research centre, which is part of the European Plant Phenotyping Network and infrastructures. Five vines per each irrigation treatment, rootstock and scion/rootstock combination were imaged before the application of drought stress (referred to as ‘Time 0’), during the drought, and during the recovery phase.

The vines were automatically transported on a conveyor belt into the two imaging chambers and identified using the barcode and RFID tracking system. Each imaging chamber was equipped with different types of illumination and cameras: broad-spectrum halogen lighting and near-infrared (NIR) camera operating in the 900-1700 wavelength range (VDS Vosskühler GmbH NIR-300P) for the NIR chamber, and fluorescent white lighting and 2 Megapixel RGB camera (Basler scA1600-28gc) for the visible light chamber.

Three images per plant were acquired from each chamber, one from the top of the plant (Top View, TV) and two from the lateral sides at an orthogonal angle (0° and 90° Side View, SV).

4.2.5 RGB-imaging

Image segmentation, which was conducted to separate the plant from the background by creating sufficient contrast, and image analysis were performed using Python v3.9 and PlantCV v3.11 open source software (Fahlgren et al., 2015).

Traits collected from the RGB images included plant projected shoot area (PSA) and solidity (S), which indicate morphometric changes, and plant color (HUE), which indicates colorimetric changes associated with green, healthy tissue and/or yellow, chlorotic tissue.

The total number of pixels corresponding to the plant area, derived from the RGB images of the two orthogonal SVs and the TV, was used to calculate the PSA, following the procedure reported by Calabritto et al. (2025). The plant S was calculated as the ratio between the plant area and the convex hull area analyzed from the orthogonal SVs. Plant color was analyzed using hue channels,

which quantify color in terms of an angle around a color hexagon with values ranging from 0° to 360°. Leaf color is usually included in the hue range from 120° (green healthy tissue) to 60° (yellow chlorotic tissue). HUE was derived from TV images of the plant to easily extract the color of the adaxial leaf surface and to reduce the influence of the stem in the hue.

4.2.6 Data analysis

All data analyses were performed in R 4.3.3 (R Studio: Integrated Development for R., Boston, MA, USA. Rcore team, 2024), with Sigmaplot 15.0 (Systat Software, Inc., San Jose, CA, USA) also used for data plotting. Physiological and phenotyping measurements were reported as means and standard errors of the means ($\pm$ SE). A two-way ANOVA was performed to determine the effects of irrigation treatment and cultivar, as well as their interaction, on physiological parameters separately for each level of water stress or recovery, after testing for normality (Shapiro-Wilk's test) and equal variance (Levene's test) hypotheses. A one-way ANOVA was used to examine the differences between irrigation treatments within each cultivar at each level of water stress or recovery. For phenotyping parameters, irrigation treatments were compared using Student's t-test after checking the normality of the data distribution. A Principal Component Analysis (PCA) was performed including physiological (gs, A and Tleaf) and phenotypic (PSA, S and HUE) variables, with the superimposition of confidence ellipses ($\alpha = 0.05$), to investigate the responses of rootstocks and scion/rootstock combinations to the least water availability achieved using a multivariate approach.

4.3 Results

4.3.1 Soil water status

SWA was progressively reduced during the imposed drought period, decreasing by about 35% FC at the maximum stress level achieved in the rootstocks and grafting combinations (Fig. 1). Three successive reductions, highlighting different water deficit conditions, were imposed on rootstocks of about 83% FC, 78% FC, and 65% FC (Fig. 1a) and on grafting combinations of about 81% FC, 77% FC, and 63% FC (Fig. 1b).

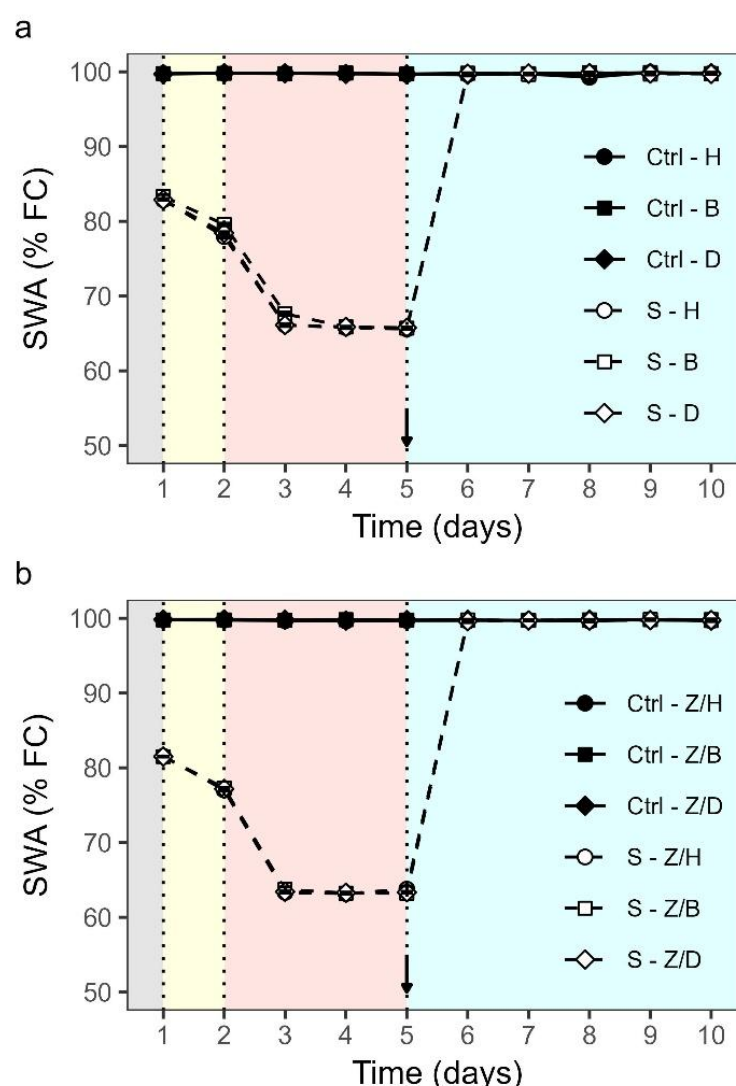


Fig. 1. Soil water availability (SWA) along the experiment for three kiwifruit (a) rootstocks ‘Hayward’ (H), ‘Bounty’ (B), and ‘D1’ (D) and (b) grafting combinations ‘Zesy002/Hayward’ (Z/H), ‘Zesy002/Bounty’ (Z/B), and ‘Zesy002/D1’ (Z/D), under well-watered conditions (Ctrl) or progressively subjected to drought (S) followed by a 5-day re-watering period. Background colors indicate different SWA reductions and stress levels, approximately: 83-81% FC (light grey); 78-77% FC (light yellow); 65-63% FC (light pink); 100% FC re-watering (light blue) in rootstocks and grafting combinations. The arrow indicates the start of re-watering. Data shown are mean $\pm$ standard error of the mean ($n=5$).

4.3.2 Leaf gas exchange response

The leaf gas exchange response of the vines was not significantly affected by irrigation treatment throughout the experiment, as shown by the results of the two-way ANOVA analysis. Values of g_s and E under drought were significantly reduced in H at the maximum SWA reduction (Fig. 2a, b). At maximum stress (i.e., 65% SWA), g_s was reduced by about 42% (in H) and 35% (in D),

dropping to values of $\sim 0.05 \text{ mol H}_2\text{O m}^{-2}\text{s}^{-1}$, compared to Ctrl vines. Values of A under drought followed the trend of g_s , with significant reductions in H at maximum stress, where values were reduced by about 46% compared to Ctrl vines (Fig. 2c). The B was unaffected by the imposed drought in terms of leaf gas exchange response, as the values of g_s , E, and A remained within the range of the Ctrl vines (Fig. 2a, b, c).

Variations in g_s in Ctrl vines were in the range of $0.08\text{-}0.14 \text{ mol H}_2\text{O m}^{-2}\text{s}^{-1}$ in H and D and of $0.11\text{-}0.16 \text{ mol H}_2\text{O m}^{-2}\text{s}^{-1}$ in B (Fig. 2a). Variations in E in Ctrl vines were in the range of $2.0\text{-}3.5 \text{ mmol H}_2\text{O m}^{-2}\text{s}^{-1}$ in H and D and of $3.2\text{-}3.6 \text{ mmol H}_2\text{O m}^{-2}\text{s}^{-1}$ in B (Fig. 2b). Variations in A in Ctrl vines were in the range of $7.0\text{-}10.8 \text{ }\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$ in H and D and of $9.7\text{-}11.7 \text{ }\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$ in B (Fig. 2c).

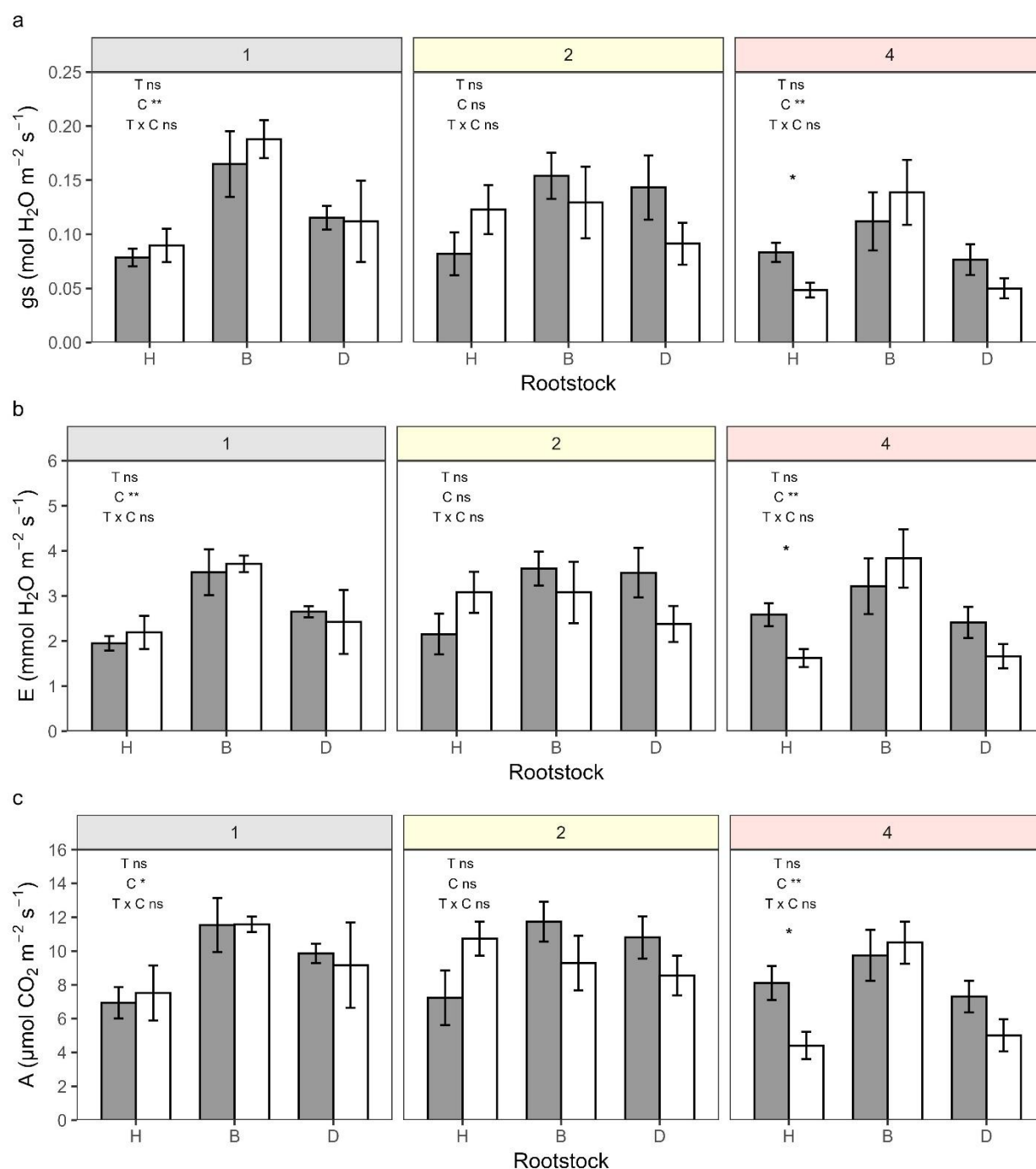


Fig. 2. Effect of drought stress on (a) stomatal conductance (gs), (b) transpiration rate (E), (c) net photosynthetic rate (A) measured for three kiwifruit rootstocks ‘Hayward’ (H), ‘Bounty’ (B), and ‘D1’ (D). During the experiment, vines were subjected to two irrigation treatments: well-watered conditions (grey bars, Ctrl, 100% FC) or progressive drought (white bars, S). Data shown are mean $\pm$ standard error of the mean ($n=4$). Two-way ANOVA p -values are shown for each parameter and level of stress. Asterisks indicate significant differences between Ctrl and S vines within each rootstock according to one-way ANOVA. Note that each panel refers to a different level of SWA for drought-stressed vines: light grey (83%), light yellow (78%), and light pink (65%). Significance codes: “*” $p<0.05$, “***” $p<0.01$, “ns” not significant. T = treatment, C =cultivar.

One day after re-watering, H recovered the g_s and A to Ctrl values (Fig. 3 a, c). D vines previously exposed to drought maintained significantly lower values at this time, but fully recovered their physiological activity 3 days after re-watering (Fig. 3a, b).

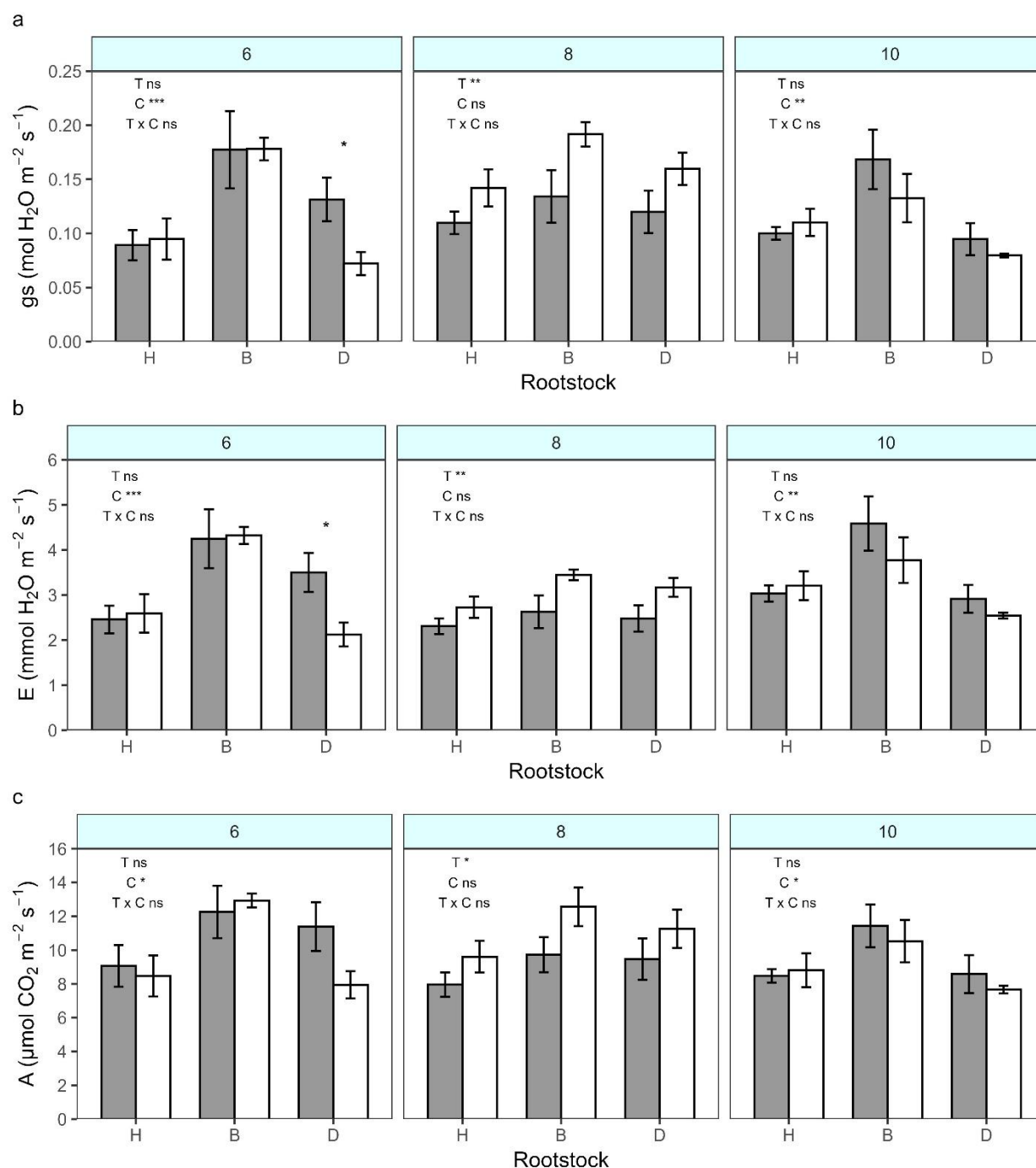


Fig. 3. Effect of re-watering on (a) stomatal conductance (g_s), (b) transpiration rate (E), (c) net photosynthetic rate (A) measured for three kiwifruit rootstocks ‘Hayward’ (H), ‘Bounty’ (B), and ‘D1’ (D). During the experiment, vines were subjected to two irrigation treatments: well-watered conditions (grey bars, Ctrl, 100% FC) or progressive drought (white bars, S). Data shown are mean $\pm$ standard error of the mean ($n=4$). Two-way ANOVA p -values are shown for each parameter and recovery day. Asterisks indicate significant differences between Ctrl and S vines within each

rootstock according to one-way ANOVA. Note that each panel refers to a different re-watering day. Significance codes: “*” $p < 0.05$, “**” $p < 0.01$, “***” $p < 0.001$, “ns” not significant. T = treatment, C =cultivar.

Leaf gas exchange activity decreased under progressive drought in all grafting combinations compared to Ctrl vines, with a significant treatment effect at the maximum level of stress (Fig. 4). Significantly lower drought-induced g_s , E , and A values were observed in Z/H at the maximum SWA reduction. At maximum stress (i.e., 63% SWA), g_s was reduced by about 66% (in Z/H), 45% (in Z/D), and 32 % (in Z/B), dropping to values of ~ 0.05 - $0.08 \text{ mol H}_2\text{O m}^{-2}\text{s}^{-1}$, compared to Ctrl vines (Fig. 4a). At this time, drought-induced E and A were in the range of 1.6 - $2.4 \text{ mmol H}_2\text{O m}^{-2}\text{s}^{-1}$ and 5.9 - $8.8 \text{ }\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$, respectively, with reductions of about 59% and 58% in Z/H, 39% and 28% in Z/D, and 26% and 17% in Z/B compared to Ctrl vines (Fig. 4b, c). Variations in g_s , E and A in Ctrl vines were in the range of 0.11 - $0.20 \text{ mol H}_2\text{O m}^{-2}\text{s}^{-1}$ (Fig. 4a), 2.3 - $4.0 \text{ mmol H}_2\text{O m}^{-2}\text{s}^{-1}$ (Fig. 4b) and 10.0 - $14.0 \text{ }\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$ (Fig. 4c), respectively.

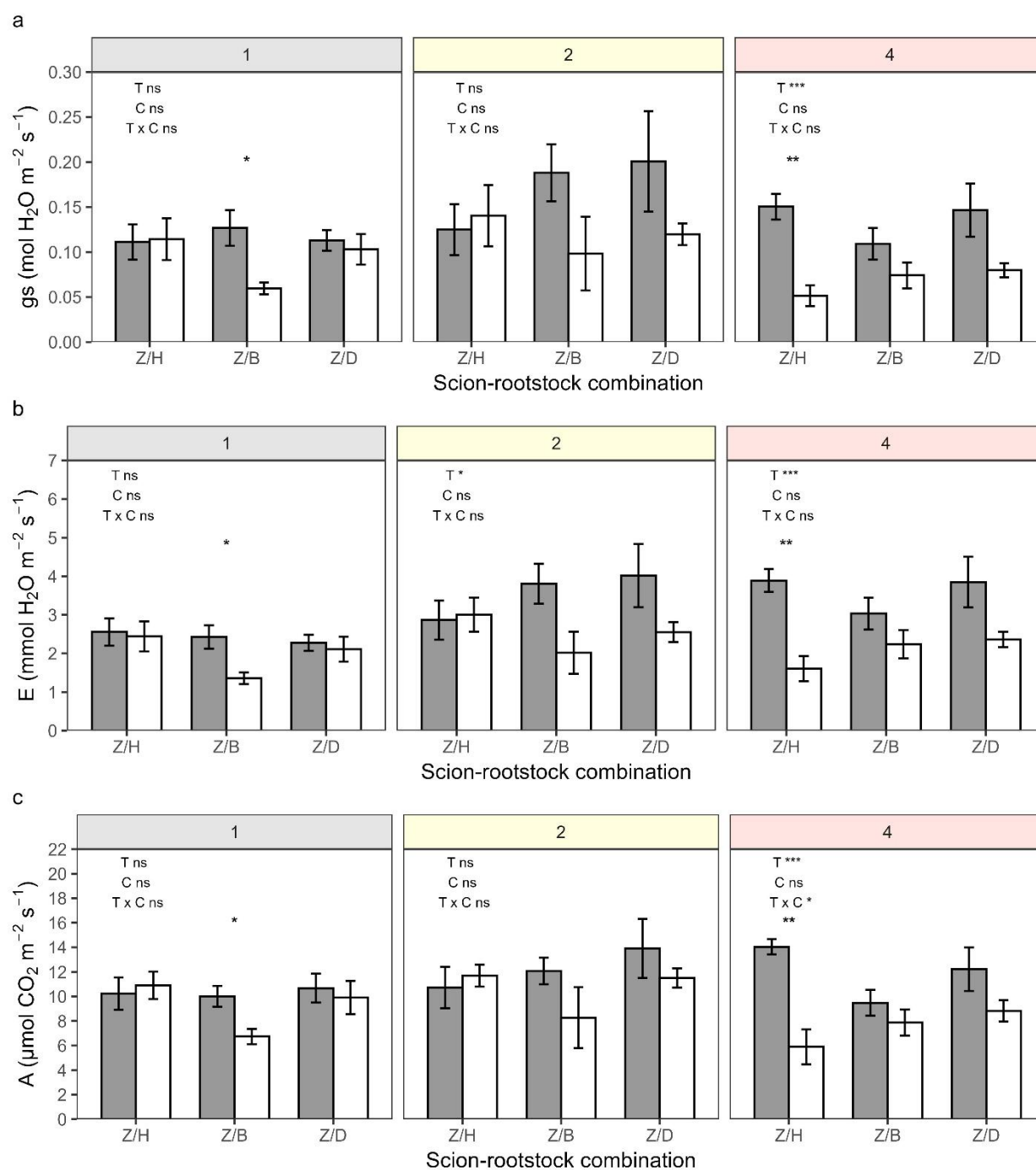


Fig. 4. Effect of drought stress on (a) stomatal conductance (gs), (b) transpiration rate (E), (c) net photosynthetic rate (A) measured for three kiwifruit grafting combinations ‘Zesy002/Hayward’ (Z/H), ‘Zesy002/Bounty’ (Z/B), and ‘Zesy002/D1’ (Z/D). During the experiment, vines were subjected to two irrigation treatments: well-watered conditions (grey bars, Ctrl, 100% FC) or progressive drought (white bars, S). Data shown are mean $\pm$ standard error of the mean ($n=4$). Two-way ANOVA p -values are shown for each parameter and level of stress. Asterisks indicate significant differences between Ctrl and S vines within each scion-rootstock combination according to one-way ANOVA. Note that each panel refers to a different level of SWA for

drought-stressed vines: light grey (81%), light yellow (77%), and light pink (63%). Significance codes: “*” $p < 0.05$, “**” $p < 0.01$, “***” $p < 0.001$, “ns” not significant. T = treatment, C =cultivar.

One day after re-watering, all grafting combinations previously exposed to drought stress showed reduced leaf gas exchange activity compared to the Ctrl vines, with a significant treatment effect, as indicated by the two-way ANOVA results. Z/D drought-stressed vines showed significantly lower values of g_s ($\sim 0.08 \text{ mol H}_2\text{O m}^{-2}\text{s}^{-1}$, Fig. 5a) and E ($\sim 2 \text{ mmol H}_2\text{O m}^{-2}\text{s}^{-1}$, Fig. 5b) than Ctrl vines. They also maintained significantly lower levels of A at this time, which was reduced by 45% compared to the Ctrl vines (Fig. 5c). Three days after re-watering, Z/H drought-stressed vines still had reduced levels of leaf gas exchange activity, although not significantly, while Z/B and Z/D stressed vines returned to Ctrl levels of g_s and A .

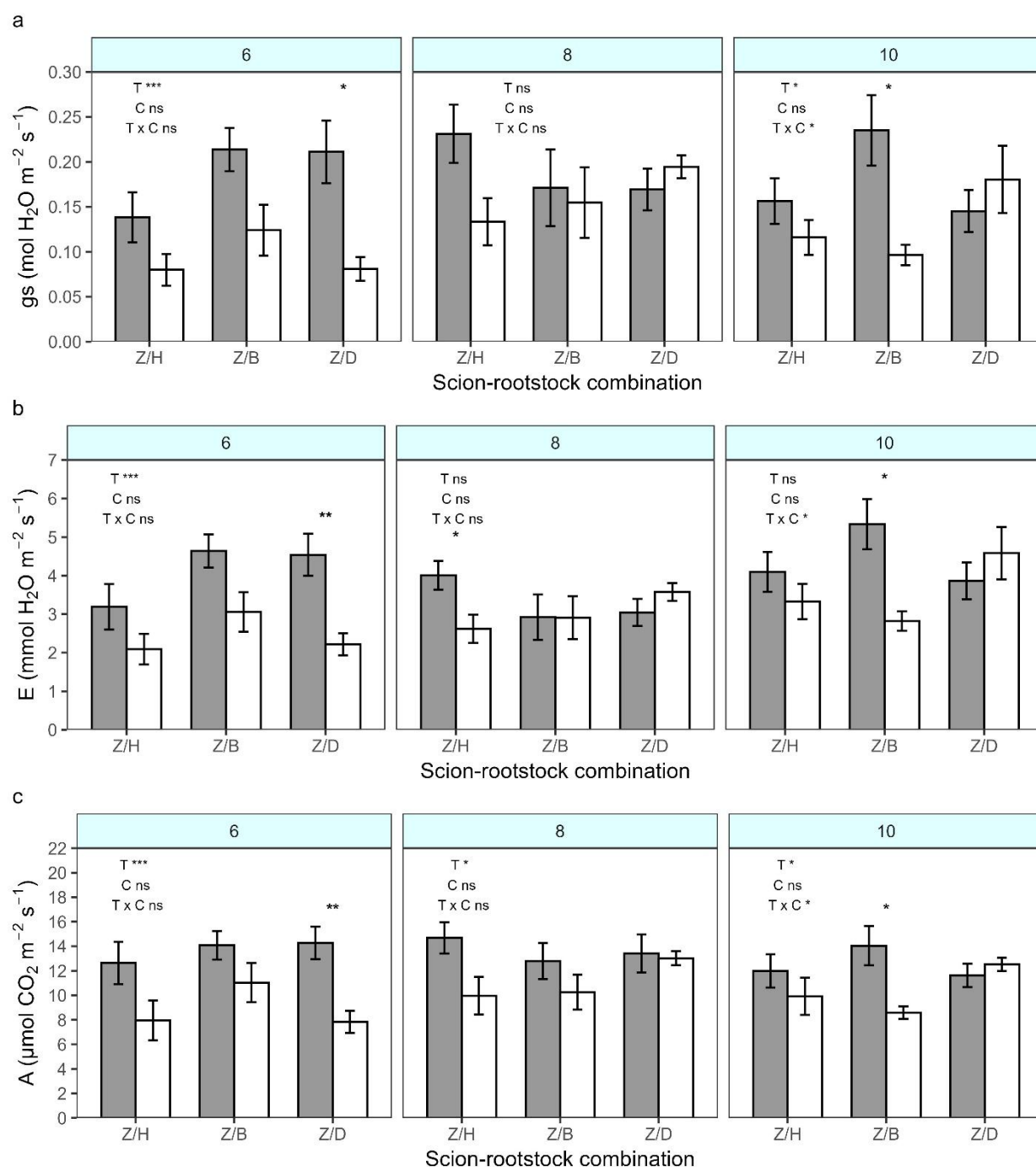


Fig. 5. Effect of re-watering on (a) stomatal conductance (gs), (b) transpiration rate (E), (c) net photosynthetic rate (A) measured for three kiwifruit grafting combinations 'Zesy002/Hayward' (Z/H), 'Zesy002/Bounty' (Z/B), and 'Zesy002/D1' (Z/D). During the experiment, vines were subjected to two irrigation treatments: well-watered conditions (grey bars, Ctrl, 100% FC) or progressive drought (white bars, S). Data shown are mean $\pm$ standard error of the mean ($n=4$). Two-way ANOVA p -values are shown for each parameter and recovery day. Asterisks indicate significant differences between Ctrl and S vines within each scion-rootstock combination according to one-way ANOVA. Note that each panel refers to a different re-watering day.

Significance codes: “*” $p < 0.05$, “**” $p < 0.01$, “***” $p < 0.001$, “ns” not significant. T = treatment, C =cultivar.

Diurnal trends of g_s and A were reported to show the behavior of rootstocks throughout the day at 65% SWA compared to Ctrl vines (Fig. 6a, b). Ctrl vines of H and D showed higher values of g_s (around $0.15 \text{ mol H}_2\text{O m}^{-2}\text{s}^{-1}$) in the middle and early morning, respectively. Then, g_s was steep (in H) and gradually decreased (in D) until the afternoon (Fig. 6a). B maintained high values of g_s even during the most critical hours (i.e., around 13:30 h), with a decrease in the afternoon (Fig. 6a). Variations in A followed those of g_s (Fig. 6b). No relevant differences were observed between Ctrl and S vines during the day, except for A in S vines of B in the afternoon, which was reduced by about 26% compared to Ctrl vines (Fig. 6b).

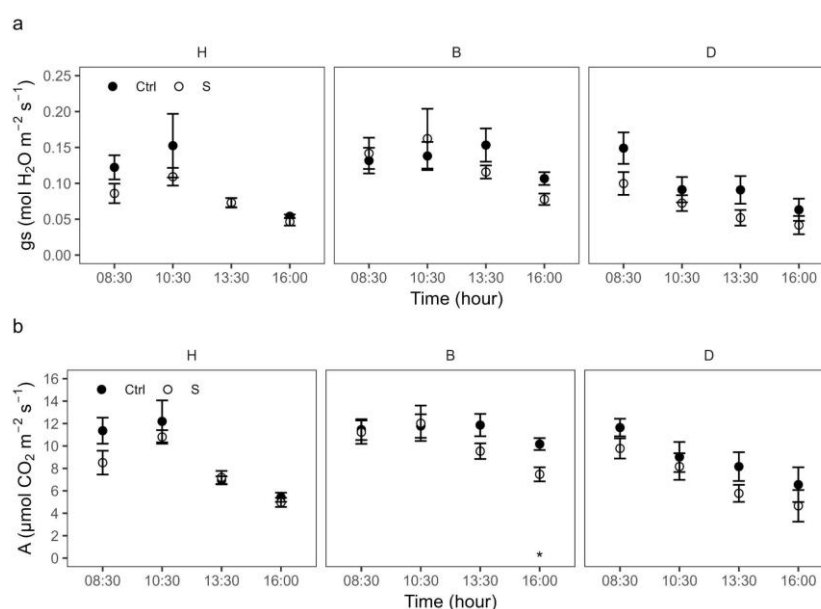


Fig. 6. Diurnal trends of (a) stomatal conductance (g_s) and (b) net photosynthetic rate (A) in three kiwifruit rootstocks ‘Hayward’ (H), ‘Bounty’ (B), and ‘D1’ (D) when SWA was reduced by 35% (Ctrl = well-watered vines at 100% FC, S = drought-stressed vines). Data shown are mean $\pm$ standard error of the mean (g_s , A $n=4$). Asterisks indicate significant differences according to Student's t-test between Ctrl and S within each time of measurement. Significance codes: “*” $p < 0.05$.

Ctrl vines of Z/H and Z/D showed higher values of g_s (around $0.14\text{-}0.16 \text{ mol H}_2\text{O m}^{-2}\text{s}^{-1}$) in the middle of the morning, then g_s gradually decreased until the early afternoon. Z/B showed the

highest value of g_s in the early morning and maintained quite high and constant values of g_s until the early afternoon (Fig. 7a).

All vines exposed to drought (63% SWA) showed reduced g_s (below $0.1 \text{ mol H}_2\text{O m}^{-2}\text{s}^{-1}$) from mid-morning and throughout the day compared to Ctrl vines, suggesting the occurrence of stomatal control. However, significantly lower drought-induced g_s values were observed in particular in Z/H and Z/D in the middle of the morning and in Z/B in the early afternoon (Fig. 7a). Variations in A followed those of g_s , with significant reductions under drought in the early afternoon in Z/B (reduced by about 58% compared to Ctrl vines) and Z/D (reduced by about 34% compared to Ctrl vines) (Fig. 7b).

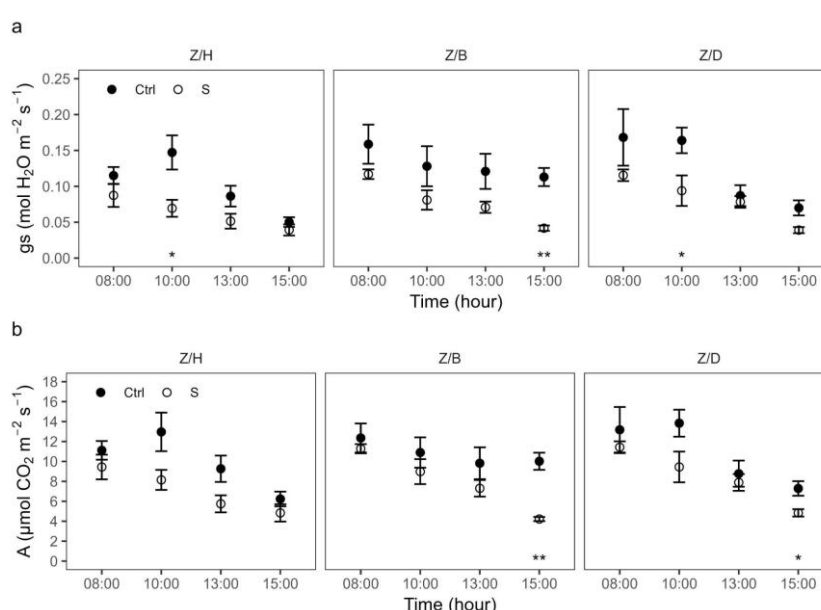


Fig. 7. Diurnal trends of (a) stomatal conductance (g_s) and (b) net photosynthetic rate (A) in three kiwifruit grafting combinations ‘Zesy002/Hayward’ (Z/H), ‘Zesy002/Bounty’ (Z/B), and ‘Zesy002/D1’ (Z/D) when SWA was reduced by 37% (Ctrl = well-watered vines at 100% FC, S = drought-stressed vines). Data shown are the mean $\pm$ standard error of the mean (g_s , A $n=4$). Asterisks indicate significant differences according to Student's t-test between Ctrl and S within each time of measurement. Significance codes: “*” $p<0.05$, “**” $p<0.01$.

4.3.3 Leaf water use and carboxylation efficiency

Photosystem II operating efficiency (Φ_{PSII}) and electron transport rate (ETR) in the light-adapted state were unaffected by a 35% reduction in SWA in any rootstock (Fig. 8a, b). Values of intrinsic water use efficiency (A/ g_s) did not vary under drought for any of the rootstocks. B showed lower values, as indicated by the significant cultivar effect from the two-way ANOVA results (Fig. 8c). The instantaneous carboxylation efficiency (A/Ci) decreased significantly due to treatment effect,

particularly in H, while it did not change in B. However, A/Ci of H and D drought-stressed vines fully restored Ctrl values at the end of the recovery period (Fig. 8d).

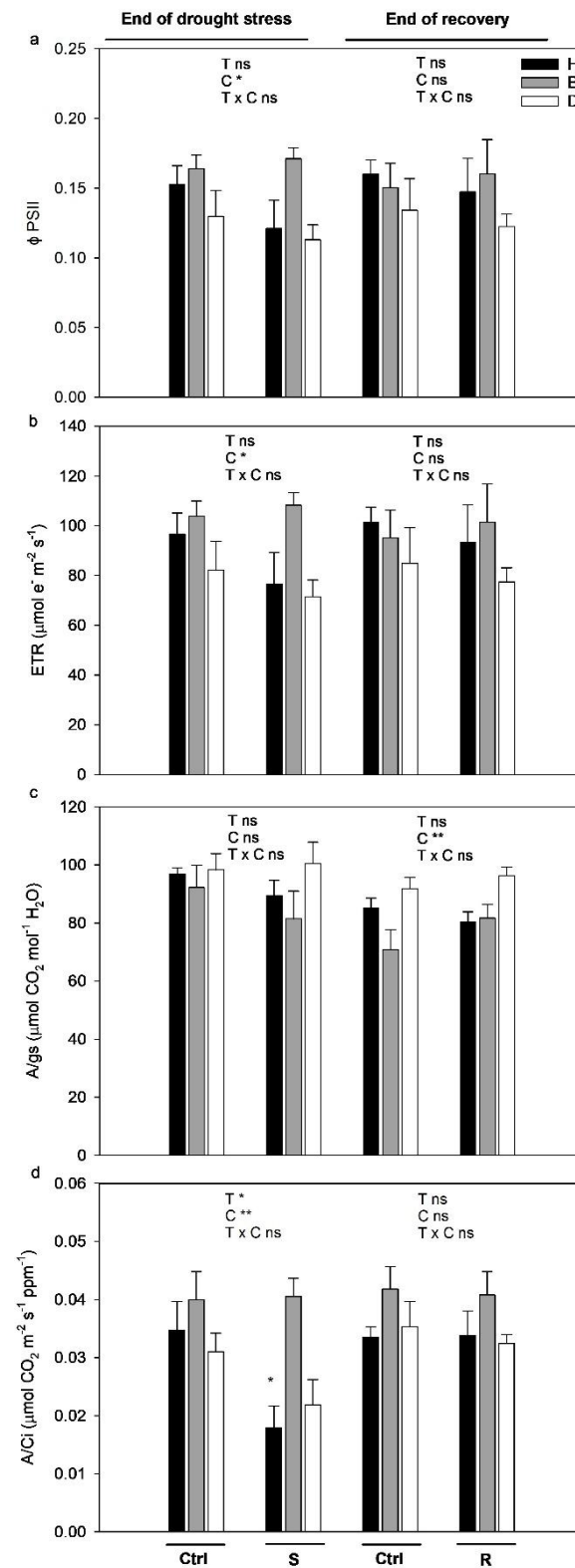


Fig. 8. Effect of drought stress and re-watering on (a) photosystem II operating efficiency (Φ_{PSII}) in the light-adapted state, (b) electron transport rate (ETR), (c) intrinsic water use efficiency (A/gs), and (d) instantaneous carboxylation efficiency (A/Ci) measured for three kiwifruit

rootstocks ‘Hayward’ (H), ‘Bounty’ (B), and ‘D1’ (D). During the experiment, vines were subjected to two irrigation treatments: well-watered conditions (Ctrl, 100% FC) or progressive drought (S), followed by a 5-day re-watering period (R). Sampling points shown were at the end of the drought stress (65% SWA) and at the end of the 5-day recovery period. Data shown are mean $\pm$ standard error of the mean ($n=4$). Two-way ANOVA p -values are shown for each parameter and stress/recovery phase. Asterisks indicate significant differences between Ctrl and S vines within each rootstock according to one-way ANOVA. Significance codes: “*” $p<0.05$, “**” $p<0.01$, “ns” not significant. T = treatment, C =cultivar.

The two-way ANOVA analysis revealed a significant influence of the treatment on Φ PSII, ETR, A/gs, and A/Ci of the grafting combinations. Φ PSII and ETR decreased significantly when SWA was reduced by 37%, with significant reductions in Z/H (Fig. 9a, b). A/gs values were found to increase in drought-stressed vines of all grafting combinations, with significant differences in Z/B and Z/D (Fig. 9c). A/Ci decreased under drought in Z/H due to the reduction in A at this time, while it did not change significantly in Z/B and Z/D (Fig. 9d). All drought-induced responses in the investigated parameters fully recovered to Ctrl levels by the end of the recovery period (Fig. 9).

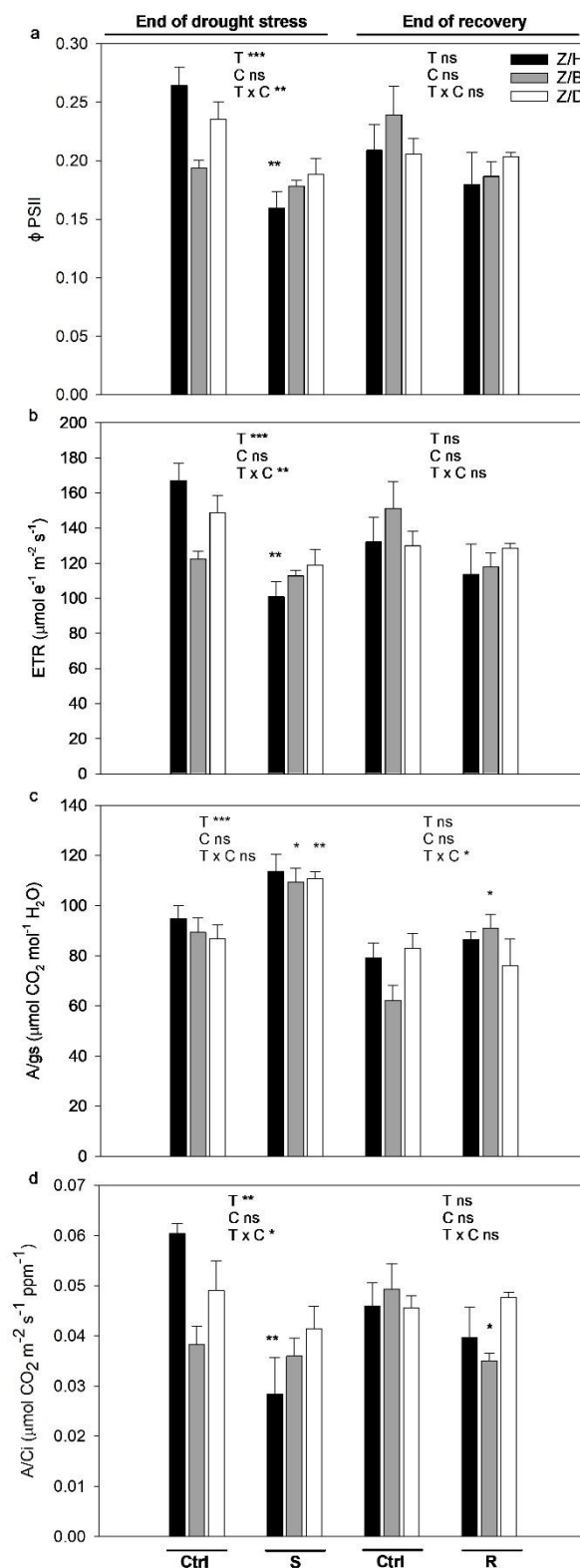


Fig. 9. Effect of drought stress and re-watering on (a) photosystem II operating efficiency (Φ_{PSII}) in the light-adapted state, (b) electron transport rate (ETR), (c) intrinsic water use efficiency (A/gs), and (d) instantaneous carboxylation efficiency (A/Ci) measured for three kiwifruit grafting combinations ‘Zesy002/Hayward’ (Z/H), ‘Zesy002/Bounty’ (Z/B), and ‘Zesy002/D1’ (Z/D). During the experiment, vines were subjected to two irrigation treatments: well-watered conditions

(Ctrl, 100% FC) or progressive drought (S), followed by a 5-day re-watering period (R). Sampling points shown were at the end of the drought stress (63% SWA) and at the end of the 5-day recovery period. Data shown are mean $\pm$ standard error of the mean ($n=4$). Two-way ANOVA p -values are shown for each parameter and stress/recovery phase. Asterisks indicate significant differences between Ctrl and S vines within each scion-rootstock combination according to one-way ANOVA. Significance codes: “*” $p<0.05$, “**” $p<0.01$, “***” $p<0.001$, “ns” not significant. T = treatment, C =cultivar.

4.3.4 RGB-imaging

The phenotypic responses of rootstocks and grafting combinations to drought were investigated by analyzing morphometric and colorimetric traits (i.e. PSA, S and HUE).

Significant relative decreases in PSA were observed only in D when SWA approached 65%, which was fully recovered soon after re-watering (Fig. 10a). S did not change significantly throughout the experiment and between Ctrl and S vines in any of the rootstocks (Fig. 10b). HUE decreased in both Ctrl and S vines over the course of the drought, with no significant differences in drought-stressed vines (Fig. 10c).

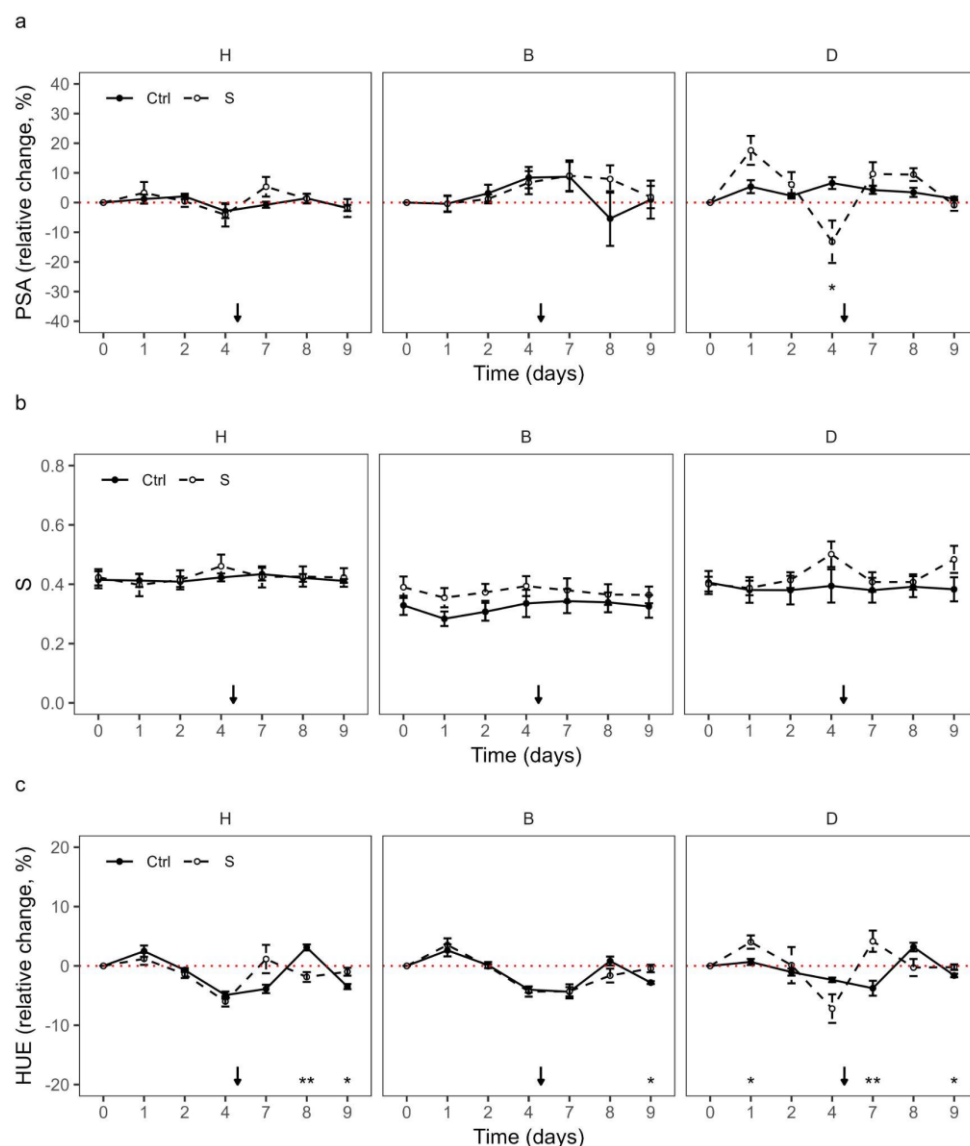


Fig. 10. Effect of drought stress and re-watering on (a) projected shoot area (PSA), (b) solidity (S), and (c) plant color (HUE) measured for three kiwifruit rootstocks 'Hayward' (H), 'Bounty' (B), and 'D1' (D). During the experiment, vines were subjected to two irrigation treatments: well-watered control conditions (Ctrl, 100% FC) or progressive drought stress (S), followed by a 5-day re-watering period. Arrows indicate the start of re-watering. Asterisks indicate significant differences according to Student's t-test between Ctrl and S within each day of measurement. Data shown are mean $\pm$ standard error of the mean ($n=5$). Note that PSA and HUE are expressed as relative changes (%) with respect to the previous measurement.

Significant relative decreases in PSA were found in both Z/H and Z/D as SWA approached 63%. Soon after re-watering, PSA increased in previously drought-exposed Z/H and Z/D vines, with values significantly different from Ctrl (Fig. 11a). S did not change significantly throughout the experiment and between Ctrl and S vines in any of the grafting combinations (Fig. 11b). HUE

decreased in both Ctrl and S vines over the course of the drought, but significantly in drought-stressed vines of Z/H (Fig. 11c).

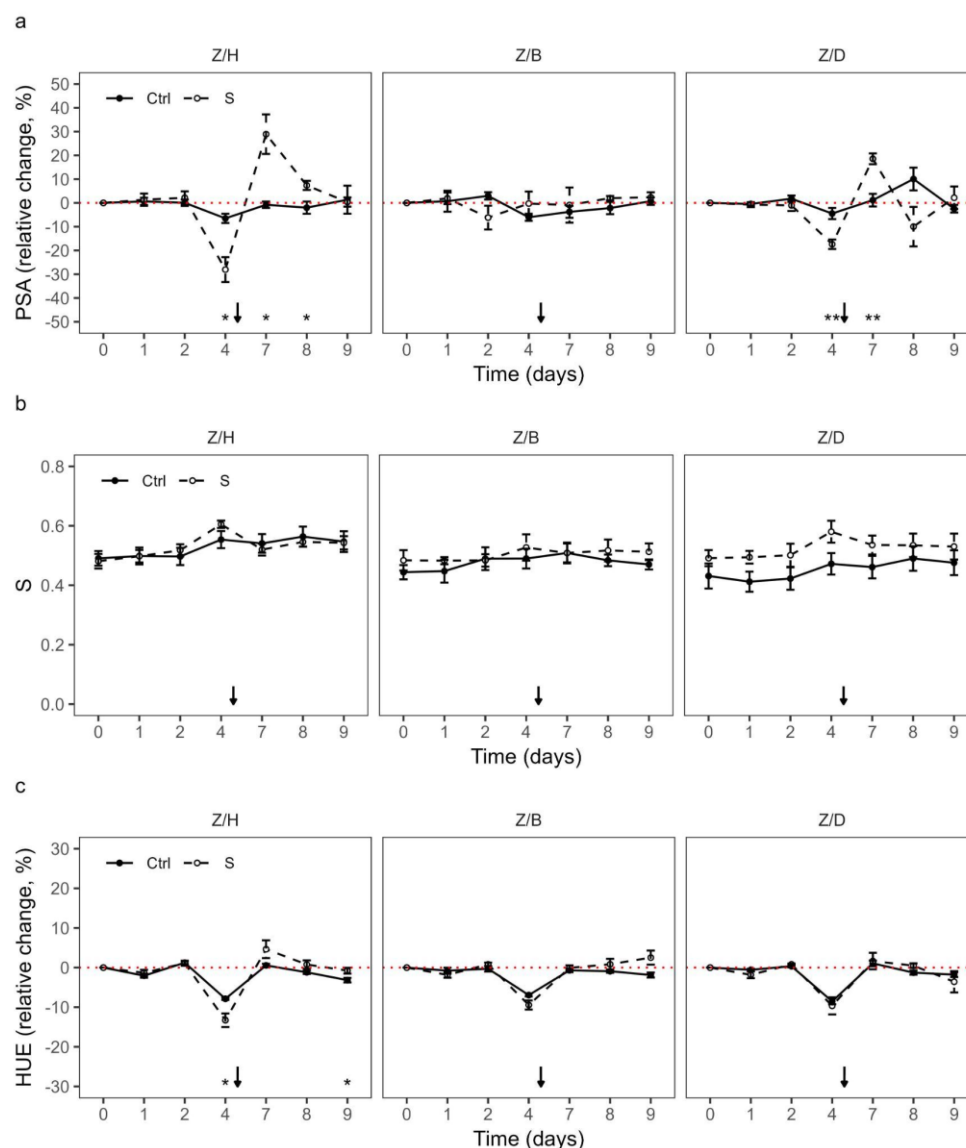


Fig. 11. Effect of drought stress and re-watering on (a) projected shoot area (PSA), (b) solidity (S), and (c) plant color (HUE) measured for three kiwifruit grafting combinations ‘Zesy002/Hayward’ (Z/H), ‘Zesy002/Bounty’ (Z/B), and ‘Zesy002/D1’ (Z/D). During the experiment, vines were subjected to two irrigation treatments: well-watered control conditions (Ctrl, 100% FC) or progressive drought stress (S), followed by a 5-day re-watering period. Arrows indicate the start of re-watering. Asterisks indicate significant differences according to Student's t-test between Ctrl and S within each day of measurement. Data shown are mean $\pm$ standard error of the mean ($n=5$). Note that PSA and HUE are expressed as relative changes (%) with respect to the previous measurement.

4.3.5 Principal component analysis

Principal component analysis (PCA) was performed to evaluate the contribution of the physiological and phenotypic variables to the variation between groups at the maximum stress level achieved. The first two principal components (Dim1, Dim2) explained approximately 81.4% and 81.2% of the total variance in rootstocks (Fig. 12a) and grafting combinations (Fig. 12b), respectively. At the maximum stress, the short distance between Ctrl and S vines of each rootstock highlighted that there was no separation by water treatment in rootstocks. However, on the x-axis (i.e. Dim1), the rootstocks tended to separate from each other (despite the water treatment applied) (Fig. 12a). Dim1 accounted for 70.7% of the variation and was mainly driven by gs and A, followed by Tleaf and phenotypic variables, indicating that increased A and gs and reduced PSA were evident between B and H rootstocks. Dim2 accounted for 10.7% of the variation and was mainly explained by HUE and PSA, followed by the physiological parameters (Fig. 12a).

For the grafting combinations analyzed at the maximum stress, the water treatments tended to separate on the x-axis of the biplot (Fig. 12b). Dim1 accounted for 56.1% of the variation and was driven by gs and A, followed by Tleaf and phenotypic variables. The left-hand side of the biplot was associated with Ctrl, with an increase in gs and A. Dim2 explained 25.1% of the variation and was driven by PSA and S, followed by HUE (Fig. 12b).

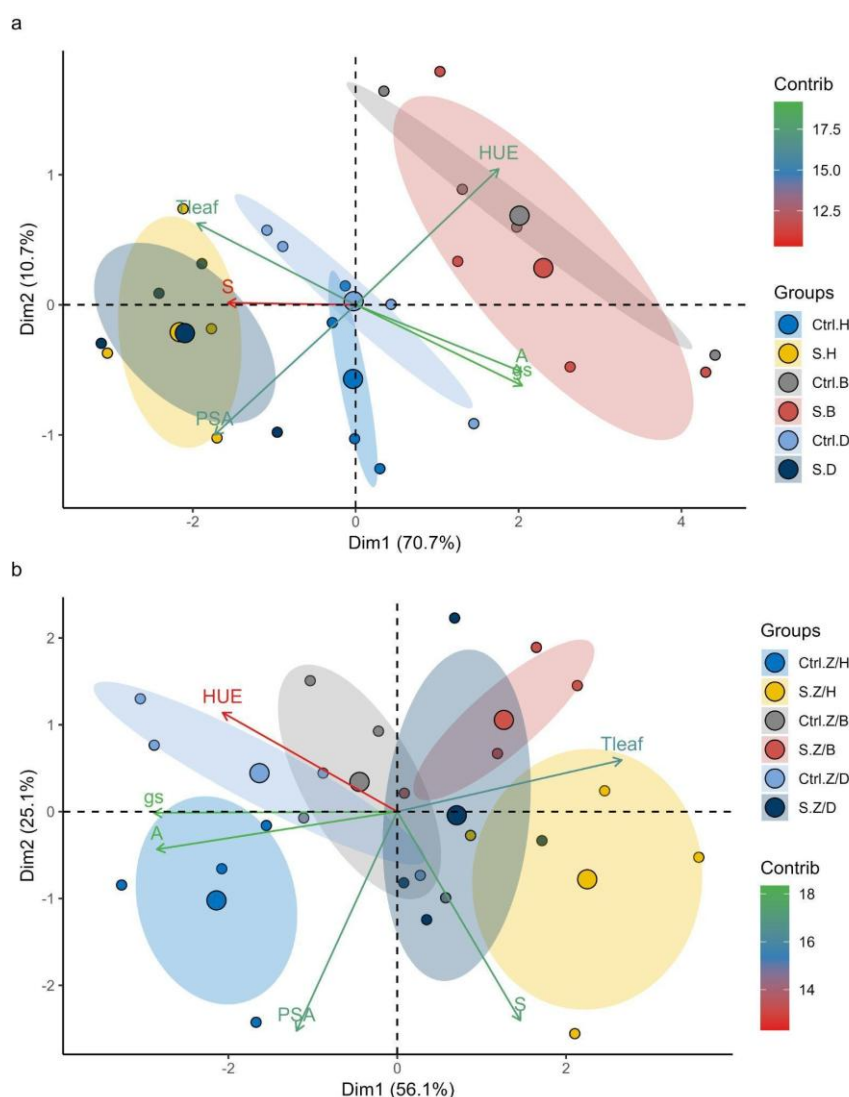


Fig. 12. Principal component analysis (PCA) of individuals based on six main physiological (gs = stomatal conductance, A = photosynthetic rate, Tleaf = leaf temperature) and phenotypic (PSA = projected shoot area, S = solidity, HUE = plant color) parameters in three kiwifruit (a) rootstocks ‘Hayward’ (H), ‘Bounty’ (B), and ‘D1’ (D) and (b) grafting combinations ‘Zesy002/Hayward’ (Z/H), ‘Zesy002/Bounty’ (Z/B), and ‘Zesy002/D1’ (Z/D), under well-watered control conditions (Ctrl) and at the maximum level of drought stress (65% and 63% SWA in rootstocks and grafting combinations, respectively) reached during the experiment (S). The proportion of the variation of the first two PCs (Dim1 and Dim2) is indicated on the axis. The direction of the arrows shows the correlations of the variables with the given PCs, while the color indicates their contribution to the PCs (gradient color). Dim = dimension, Contrib = contribution.

4.4 Discussion

Leaf gas exchange measurements on kiwifruit rootstocks and scion/rootstock combinations under progressive drought and after re-watering, coupled with phenotypic analysis, revealed differential

responses among the genotypes studied: i) drought stress imposed by progressively reducing pot soil water availability had no effect on the morpho-physiological responses of B rootstock and only minor effects in D and H, with complete recovery after re-watering; ii) all scion/rootstock combinations were affected by drought to different degrees. Reduced values of g_s and A were observed in all scion/rootstock combinations under drought (-66% and -58% in Z/H, -45% and -28% in Z/D, -32% and -17% in Z/B). Z/H was the most sensitive to drought, as indicated by significant decreases in physiological parameters and PSA and HUE at the end of the drought stress, followed by Z/D. Z/B, although showing a reduction in g_s under drought, was not affected in photosynthetic and phenotypic parameters, suggesting its lower sensitivity to drought; iii) at maximum drought stress, rootstocks were not separated by water treatment but by genotype, highlighting the great diversity of genetic material and the lack of influence of imposed drought stress. Instead, scion/rootstock combinations were separated by water treatment, based on physiological and phenotypic variables.

The progressive reduction in pot water availability imposed in the experiment resulted in a mild drought compared to another study that examined the drought response of different kiwifruit genotypes at 40-45% FC (moderate drought) and 25-30% FC (severe drought) (Bao et al., 2024). According to the purpose of this study, the imposed stress period was not so long and severe as to cause detrimental effects on the physiological and phenotypic parameters of the vines, in order to simulate the short-term or intermittent drought conditions that can easily occur due to the challenges of climate change (critical atmospheric conditions in summer combined with water shortage).

According to the two-way ANOVA analysis, the treatment had no or only minor effects on the investigated parameters of rootstocks, with the cultivar instead having a prevailing effect. On the contrary, a significant and prevailing treatment effect was found at the end of the drought and at the start of the recovery period in the scion/rootstock combinations. The results showed that g_s was sensitive to soil water availability and decreased under drought in all scion/rootstock combinations. Drought-induced changes in A were consistent with those in g_s , reaching significant reduction in Z/H as SWA was further reduced to the minimum level reached around 63% FC. Previous studies have reported a significant and immediate decrease in g_s after the imposition of drought in kiwifruit (Gucci et al., 1996; Wang et al., 2011; Wu and Zhu, 2022; Bao et al., 2024). On a diurnal basis, both A and g_s of drought-stressed vines were higher in the morning and decreased during the day, as previously reported for pot-grown kiwifruit (Gucci et al., 1996). In particular, g_s responded more rapidly to reduced irrigation than A , which began to be limited in

all scion/rootstock combinations when g_s dropped below $0.10 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$, as found by Mills et al. (2009).

Concurrent with the consistent decrease in A (at the maximum SWA reduction reached), ETR and ΦPSII were reduced in Z/H followed by Z/D, whereas no differences were observed in Z/B under drought. These results suggest that the photosynthetic metabolism of all scion/rootstock combinations was progressively down-regulated with increasing water stress, but based on a different stage of inhibition of photosynthesis (Flexas et al., 2004). In particular, Z/B showed a slower decrease in g_s and A under drought, without inducing variations in ETR and ΦPSII . Conversely, Z/H reached a successive stage where ETR and ΦPSII were significantly affected by drought imposition at the end of the experiment, suggesting the involvement of metabolic limitations. In addition to stomatal limitation (reduced g_s), reduced ETR, ΦPSII and A/C_i are important limiting factors in A , which were observed for Z/H among the combinations studied. However, the maintenance of A values under mild drought resulted in an increased A/g_s ratio in all scion/rootstock combinations, which was significant in Z/B and Z/D compared to Ctrl vines, suggesting the adoption of a water-saving strategy and that stomatal closure was the dominant limitation to A (Montanaro et al., 2007; Sapeta et al., 2013). The increase in A/g_s is a direct consequence of the reduction in g_s before A , which was previously observed and recognized as a typical response of plants to mild drought (Flexas et al., 2010; Sapeta et al., 2013; Talbi et al., 2020).

Under mild to moderate drought stress, the decrease in A is mainly due to a decrease in g_s , and the recovery of gas exchange parameters after re-watering is generally quite rapid, with a tendency to return to initial levels after rehydration (Flexas et al., 2004). However, the level of recovery varies with the degree of previous water stress reached (Gucci et al., 1996; Miyashita et al., 2005). In all scion/rootstock combinations, g_s and A recovered after re-watering, restoring Ctrl values one and three days after re-watering in Z/H and Z/D, respectively.

Reduced soil water availability causes physiological changes in kiwifruit when there are no significant changes in plant morphology, suggesting that an initial drought response occurs in the physiological characteristics and only later in the morphometric parameters of the vine (Wu and Zhu, 2022). Drought resulted in a reduction of PSA in both Z/H and Z/D at the end of the experiment, which returned to Ctrl values after re-watering, suggesting a reversibility of both morphological and physiological changes induced by drought. The advantages and potential of HTPP to discriminate between water treatments and its applicability to characterize plant drought

responses are usually related to cereal or vegetable crops. Previous studies have reported reduced above-ground biomass, and therefore reduced projected area, together with changes in color traits under drought, highlighting the ability of HTPP to non-destructively assess temporal responses of plants to different water treatments (Kim et al., 2020; Tolley et al., 2020, Genangeli et al., 2023).

Based on changes in leaf gas exchange activity and morphometric and colorimetric parameters, the *A. macrosperma* genotype was more resilient to drought stress than the other kiwifruit species studied (*A. chinensis* var. *deliciosa*). Root system growth and hydraulic conductivity can be influenced by the rootstock and are important factors in improving water uptake and transport (Clearwater et al., 2004; Gambetta et al., 2012; Serra et al., 2014). It can be hypothesized that the increased tolerance to drought of *A. macrosperma* is likely linked to a higher root-to-shoot ratio, and that increased root development and surface area, together with anatomical features that enhance conductivity, improve overall water uptake and transport to the shoots. Alsina et al. (2011) associated increased root growth and root system conductance with drought tolerance in grapevines. A previous study comparing the physiological and growth responses of five *Actinidia* species to drought stress under tissue culture conditions showed that *A. macrosperma* is indeed a more resilient germplasm resource than *A. chinensis* var. *deliciosa* and represents a promising kiwifruit breeding material (Zhong et al., 2018). However, the drought tolerance of this species should be further investigated under field conditions. This result is not consistent with Bao et al. (2024), who reported that *A. macrosperma*, together with ‘Hayward’, is among the most sensitive genotypes to drought and could hardly recover after re-watering.

A comprehensive approach, including both phenotypic and physiological components, was used in the present study and should be established for the accurate assessment of abiotic stress sensitivity in fruit tree crops. On the basis of six physiological (A, gs and Tleaf) and phenotypic (PSA, S and HUE) indicators, all scion/rootstock combinations, unlike non-grafted rootstocks, tended to differentiate under drought stress relative to Ctrl vines. The scion grafted on ‘Hayward’ rootstock showed the highest sensitivity to drought stress. The results of the PCA analysis showed that the most important variables to explain a PC axis included both physiological and phenotypic traits, reinforcing the role of HTPP in understanding the behavior of different genotypes under water deficit.

Kiwifruit production should be supported by accurate irrigation management and plant breeding to meet the current climate challenges. Further efforts should be made to select rootstocks based

on traits that ensure better water use efficiency and increase vine tolerance to temporary droughts and other abiotic stresses that are likely to occur under climate change.

4.5 Conclusions

Heat waves and droughts are predicted to become more intense and frequent in many regions in the coming years due to climate change, with severe impacts on the Mediterranean basin. In this study, a combined approach based on physiological and phenotypic analysis was used to evaluate the response of different kiwifruit rootstocks and scion/rootstock combinations to drought stress. The three rootstocks compared here showed no marked morpho-physiological differences during mild drought. However, at the end of the drought, H and D showed an initial impairment of physiological parameters, whereas B remained unaffected, suggesting its lower sensitivity to drought. As pot water availability was progressively reduced, scion/rootstock combinations were affected by drought to different degrees, with A starting to be reduced when SWA approached 63% FC due to reduced g_s (below $0.10 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$). All genotypes were able to cope with the drought conditions imposed during the experiment, as shown by a good recovery capacity after re-watering in gas exchange, photosynthetic and phenotypic parameters. However, Z/B was identified as the least sensitive to drought stress, while Z/H was recognized as the most sensitive to the drought conditions imposed during the experiment. As abiotic stresses are expected to threaten crop production, investigating the functional response of currently used kiwifruit rootstocks to drought stress and understanding scion/rootstock interactions in response to mild and intermittent drought can support breeding and selection programs. In this regard, the combined approach of physiological and phenotypic analysis could be an effective tool to screen for more drought-tolerant kiwifruit genotypes.

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5. Physiological and image-based phenotyping assessment of waterlogging responses of three kiwifruit rootstocks and grafting combinations

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Abstract

Kiwifruit species have a relatively high rate of root oxygen consumption, making them very vulnerable to low root zone oxygen concentrations resulting from soil waterlogging. Recently, kiwifruit rootstocks have been increasingly used to improve biotic and abiotic stress tolerance and crop performance under adverse conditions. The aim of the present study was to evaluate morpho-physiological changes in kiwifruit rootstocks and grafting combinations under short-term waterlogging stress. A pot trial was conducted at the ALSIA PhenoLab, part of the Phen-Italy infrastructures, using non-destructive RGB and NIR image-based analysis and physiological measurements to identify waterlogging stress indicators and more tolerant genotypes. Three pot-grown kiwifruit rootstocks ('Bounty 71', *Actinidia macrosperma* - B; 'D1', *Actinidia chinensis* var. *deliciosa* - D; 'Hayward', *A. chinensis* var. *deliciosa* - H) and grafting combinations, with a yellow-fleshed kiwifruit cultivar ('Zesy002', *A. chinensis* var. *chinensis*) grafted on each rootstock (Z/B, Z/D, Z/H), were subjected to a control irrigation treatment (WW), restoring their daily water consumption, and to a 9-day waterlogging stress (WL), based on substrate saturation. Leaf gas exchange, photosynthetic activity, leaf temperature, RGB and NIR data were collected during waterlogging stress. Stomatal conductance and transpiration reached very low values (less than $0.05 \text{ mol m}^{-2} \text{ s}^{-1}$ and $1 \text{ mmol m}^{-2} \text{ s}^{-1}$, respectively) in both waterlogged D and H rootstocks and their grafting combinations. In turn, leaf temperature was significantly increased and photosynthesis was reduced ($1\text{-}6 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$) from the first days of waterlogging stress compared to B rootstock and combination. The B rootstock showed prolonged leaf gas exchange and photosynthetic activity, indicating that it can cope with short-term and temporary waterlogging and improve the tolerance of grafted kiwi vines, which showed a decrease in stomatal conductance five days after the onset of stress. Morphometric and colorimetric parameters from the image-based analysis confirmed the greater susceptibility of D and H rootstocks and their grafting combinations to waterlogging stress compared to B. The results presented confirm the role of physiological measurements and enhance that of RGB and NIR images in detecting the occurrence of water stress and identifying more tolerant genotypes in kiwifruit.

5.1 Introduction

Kiwifruit is a very important fruit crop in Italy, which is actually the world's third-largest producer, with 24,040 ha and annual production of 523,120 tons (FAOSTAT, 2022), gaining commercial importance and playing a key role in kiwifruit trade dynamics (Mian et al., 2022a). Recently, the introduction of new commercially grown kiwifruit cultivars has increased in the yellow-fleshed category (Burdon et al., 2014), reducing the choice of the most popular green cultivars. Kiwifruit vines are particularly exposed and sensitive to climatic conditions (Cradock-Henry, 2017) and to biotic (Vanneste, 2017) and abiotic stresses [e.g., water stress (Xiloyannis et al. 2023) and salinity stress (Abid et al., 2020)]. In recent years, increased solar radiation and summer temperatures and irregular rainfall distribution, both exacerbated by climate change, have posed new climatic challenges for kiwifruit cultivation (Testolin and Ferguson, 2009). Research and selection of new genetic material (rootstocks and cultivars) is essential to meet the new challenges posed by changing environmental conditions, with an increase in extreme events and the emergence of stresses.

Since kiwifruit cultivation has received particular attention, physiological disorders have been observed in orchards around the world as a long-term consequence of flooding events (Reid et al., 1991). In Italy, kiwifruit physiological disorders have been reported since 2012 and have been referred to as the 'Kiwifruit Vine Decline Syndrome' (KVDS), which is seriously threatening many cultivated areas, rapidly spreading across northern and central Italy (Bardi, 2020). Although a multifactorial origin of this syndrome has been suggested (Donati et al., 2020; Bardi et al., 2022), waterlogging and asphyxiating conditions represent triggering factors for the expression of KVDS (Bardi, 2020; Donati et al., 2020; Savian et al., 2020; Spigaglia et al., 2020; Sofo et al., 2022; Di Biase et al., 2023; Prencipe et al., 2023; Sofo et al., 2024).

Waterlogging stress can be injurious or even lethal to crops as water saturates the root environment, reducing oxygen availability, affecting nutrient and water uptake, and promoting a shift to the anaerobic metabolism (Sairam et al., 2008), which lead to potential economic loss in fruit tree crops (Li et al., 2021). Waterlogging conditions easily occur in kiwifruit orchards due to extreme meteorological events and improper and empirical irrigation management (Bardi, 2020; Sofo et al., 2024), exacerbated by heavy and poorly drained soils characterized by clay-loam to clay textures and low organic matter content (Domingo et al., 2002). Frequent or long-term waterlogging conditions also lead to a more or less gradual deterioration of soil structure and quality and the establishment of anoxic conditions (Sofo et al., 2022, 2024). Plant growth is significantly compromised by oxygen deficiency or lack, and roots only grow in small areas of more oxygenated soil, greatly reducing the potential for root exploration of the soil volume under

aerated conditions (Sairam et al., 2008). Previous studies demonstrated that kiwifruit has a high-water requirement (Holzapfel et al., 2000) but, at the same time, is extremely vulnerable to waterlogging and low oxygen concentrations in the root zone, which have a detrimental effect on plant growth and stomatal activity (Savé and Serrano, 1986; Smith et al., 1989, 1990) being one of the most waterlogging intolerant fruit trees (Bai et al., 2019). Plant responses to waterlogging and oxygen deprivation involve a wide range of metabolic, hormonal, and morphological adaptation and physiological and antioxidative defense processes (Domingo et al., 2002; Sairam et al., 2008; Li Z. et al., 2021; Zahra et al., 2021).

In fruit tree crops, resistance to waterlogging and consequent oxygen deficiency can be influenced by the characteristics of the rootstock (Domingo et al., 2002), which can activate several mechanisms to delay the abiotic stress-induced effects (Fullana-Pericàs et al., 2020).

Changes in environmental factors due to global climate change and the specific physiological traits and anatomy of kiwifruit vines pose emphasis on accurate orchard management and suitable techniques to avoid reductions in kiwifruit production areas, which are severely threatened by the rapid spread of physiological decline, and to increase new plantings in different climate scenarios. In order to promote the expansion in regions with soil and other environmental challenging factors, grafting kiwifruit cultivars on rootstocks with resistance or tolerance characteristics to different soil stresses could be of great importance (Mian et al., 2022b). All available measures, from the adoption of the best agronomic practices to avoid the establishment of waterlogging conditions to the identification of tolerant rootstocks, should be implemented to address the current challenges. Although genetic and breeding programs have increased the number of cultivars in recent years (Mian et al., 2022a), few rootstocks are currently commercially available for kiwifruit propagation (Clearwater et al., 2004; Li D. et al., 2021). The main cultivated kiwifruit cultivars, from *Actinidia chinensis* var. *deliciosa* and *A. chinensis* var. *chinensis*, are typically grafted onto *A. chinensis* var. *deliciosa* seedlings for commercial planting (Friend et al., 2014; Li D. et al., 2021).

Previous studies focused on the physiological responses of *Actinidia* genotypes to waterlogging stress, including some rootstocks used for kiwifruit cultivation, with the aim of identifying tolerant genotypes and describing waterlogging tolerance mechanisms (Li et al., 2020, Li Z. et al., 2021). Among these, *A. chinensis* var. *deliciosa* (cv ‘Hayward’) has been reported as a sensitive rootstock to waterlogging stress, showing a rapid decrease in stomatal conductance, transpiration and net photosynthetic rate with severe damages to the leaves and root system a few days after waterlogging stress application (Li Z. et al., 2021; Kataoka et al., 2021; Beppu et al., 2022). In contrast, *Actinidia macrosperma* has a greater tolerance to waterlogging conditions, maintaining relatively high levels of leaf gas exchange and photosynthetic activity for a longer time under

waterlogging stress, with rare leaf burn and defoliation processes (Beppu et al., 2022), whose tolerance has also been reported in the kiwifruit scion grafted on it (Kataoka et al., 2021).

Although several studies have been carried out to evaluate the molecular and physiological responses to waterlogging, phenotyping for waterlogging tolerance has been poorly addressed (Langan et al., 2022). Image-based phenotyping methods allow the assessment of shoot and root traits, such as shoot biomass and morphometry, photosynthesis-related traits, root biomass and architecture, with both advantages and challenges to detect and improve the characterization of waterlogging tolerance among crop species (Langan et al., 2022).

In the present research, a phenotyping approach based on the identification of both morphometric and colorimetric traits and indicators, accompanied by monitoring of physiological parameters, is used for the first time in kiwifruit to improve the assessment of waterlogging response. A short-term waterlogging experiment was conducted a) to assess the tolerance/susceptibility of different kiwifruit rootstocks to waterlogging stress based on physiological and phenotyping analysis, b) to evaluate the effect of the rootstock on the responses of the grafted yellow-fleshed kiwifruit cultivar to waterlogging, and c) to define a methodology integrating physiological and phenotyping indicators to screen for more tolerant genotypes to abiotic stress.

5.2 Materials and methods

5.2.1 Plant material and growth conditions

The experimental trial was conducted at the ALSIA ‘Metapontum Agrobios’ Research Centre in Metaponto (South Italy) during the 2023 growing season. A total of 30 two-year-old self-rooted vines of ‘Bounty 71’ (B) (*A. macrosperma*), ‘D1’ (D) (*A. chinensis* var. *deliciosa*), and ‘Hayward’ (H) (*A. chinensis* var. *deliciosa*) and 42 one-year-old scions of *A. chinensis* var. *chinensis* (cv ‘Zesy002’, also known as ‘G3’) grafted on each rootstock (Z/B, Z/D and Z/H) were used in the experiment. All kiwifruit vines were transplanted in March into 3 L pots filled with a mixture of acid peat and pumice (pH 6, bulk density 0.23 g cm⁻³, and 90% porosity). The vines were grown in an unheated and unconditioned greenhouse under natural light conditions and randomly distributed to reduce the effect of possible microclimatic variations in the greenhouse through spatial distribution. All vines were trained with a single main shoot and tied to a wooden stick support painted blue to allow image acquisition, segmentation, and subsequent data analysis. Vines were pruned to a similar leaf area of approximately 10 leaves each. Each pot was identified by a barcode to allow the platform to automatically read and identify the vines during image acquisition. From transplanting until the beginning of the experimental trial, all vines were fully irrigated according to evapotranspiration demands.

5.2.2 Experimental design and irrigation treatments

Before the beginning of the experimental trial, the reference pot weight, corresponding to the field capacity (FC), was determined by fully watering each pot and then allowing the excess water to drain until a stable pot weight was reached. On 10 July, five vines from each rootstock and seven from each scion-rootstock combination were subjected to waterlogging stress, while an equal number of each rootstock and scion-rootstock combination continued to be optimally irrigated. In particular, the well-watered (WW) vines were optimally irrigated by maintaining the soil water content of the pots close to FC, replenishing water lost during the day by automatically weighing the pots three times a day and restoring the reference weight at FC. The waterlogging (WL) treatment was carried out by manually overwatering the vines. For nine consecutive days of waterlogging stress (referred to as days after waterlogging, DAW) the substrate was kept continuously saturated. Each vine was placed in a plastic container filled with water to a height of 1/3 of the pot to simulate soil conditions, which are one of the factors that trigger physiological decline in the field.

5.2.3 Leaf gas exchange measurements

Stomatal conductance (gs) and transpiration (E) were measured during the day at approximately 3-h intervals for rootstocks (9:00, 11:00, 14:00, and 16:00 h, solar time) and scion-rootstock combinations (8:00, 10:00, 13:00, and 15:00 h, solar time) at 1, 2, 3, and 4 DAW and at the hours of maximum efficiency (10:00-12:00 h) at 5, 7, and 9 DAW using a portable handheld LI-600 porometer system integrated with a fluorometer (Li-Cor Biosciences, Lincoln, USA). Photosynthesis (A), internal CO₂ concentration (Ci), and leaf temperatures (T_{leaf}) were measured during the day at approximately 3-h intervals for rootstocks (9:00, 11:00, 14:00, and 16:00 h, solar time) and scion-rootstock combinations (8:00, 10:00, 13:00, and 15:00 h, solar time) at 1, 2, 3, and 4 DAW using a portable photosynthesis system Li-Cor 6800 (Li-Cor, Inc., Lincoln, NE, USA). Leaf gas exchange measurements were taken at an ambient (CO₂) of 400 µmol mol⁻¹, with temperature and external ambient photosynthetic photon flux density (PPFD) maintained at the prevailing external environmental conditions and the operating flow rate set at 500 µmol s⁻¹. To minimize the potential effects of leaf position and developmental age, all the gas exchange measurements were taken on a fully expanded and exposed leaf, with no visual symptoms of stress, from the mid-shoot region of four randomly selected vines for each irrigation treatment, rootstock, and scion-rootstock combination. The intrinsic water-use efficiency was calculated as WUEi = A/gs (Bellasio, 2023).

5.2.4 High-throughput plant phenotyping

Phenotyping measurements, based on the morphological and physiological characterization of the vines, were carried out non-destructively through plant imaging. Five vines per irrigation treatment from each rootstock and scion-rootstock combination were imaged at 1, 2, 3, 4, 7, and 9 DAW using a Scanalyzer 3D plant phenotyping platform (LemnaTec GmbH, Aachen, Germany), which is part of the Phen-Italy platform located at ALSIA ‘Metapontum Agrobios’. The vines were automatically transported by a conveyor belt into the two imaging chambers with two types of illumination, broad-spectrum halogen lighting for the near-infrared (NIR) chamber and fluorescent white lighting for the visible light (RGB) chamber, and identified using a barcode and RFID tracking system. The NIR chamber was equipped with NIR cameras operating in the 900-1,700 wavelength range (VDS Vosskühler GmbH NIR-300P), and the images were used to assess the water content of plant tissues, as reported in Petrozza et al. (2014). The visible light chamber for RGB imaging was equipped with cameras with a resolution of approximately 2 megapixels (Basler scA1600-28gc). The images were used to evaluate the health status and stress responses of the vines by analyzing color (i.e., green color corresponding to healthy tissue and degree of yellowing indicative of chlorotic tissues) and morphometric parameters such as projected shoot area and solidity. Three images were acquired from each chamber, one from the top of the plant (top view, TV) and two from the lateral sides at an orthogonal angle (0° and 90° side view, SV), to provide an average plant area correction for possible leaf overlap and then a robust representation of the total plant area (Golzarian et al., 2011). NIR acquisitions were taken at 2, 3, and 9 DAW and simultaneously with RGB images.

5.2.5 Image analysis

Image segmentation, which separated the plant from the background by creating sufficient contrast, and subsequent image analysis were performed using Python v3.9 and PlantCV v3.11 open-source software (Fahlgren et al., 2015). In particular, the sum of the number of pixels corresponding to the plant object area, derived from the RGB images of the two orthogonal SVs and the TV, was converted into cm² using a calibration factor (Golzarian et al., 2011) and referred to as the projected shoot area (PSA), determined following a procedure similar to that reported by Genangeli et al. (2023):

$$PSA = \text{Area(TV)} + \text{Area}(0^\circ\text{SV}) + \text{Area}(90^\circ\text{SV}) \quad (1)$$

Where the result is the sum of the three target plant areas from the top and from the side views expressed in cm².

How much of the hull area of the plant is covered by the leaves was identified through the solidity (S) and calculated as the ratio of the plant pixel area to the convex hull shape pixel area containing all plant pixels from plant images (Petrozza et. al., 2023).

RGB images were then analyzed with the hue component, which corresponds to an angular position around a central point, scaled in degrees from 0° to 360°, with different degrees indicating different colors. Leaf and total plant color were obtained by calculating the weighted mean value from the histogram of the hue channel in the hue saturation value (HSV) color space, resulting in a value from 0° to 360° (Petrozza et al., 2023). Usually, leaf color is included in the hue range from 120° (green — healthy tissue) to 60° (yellow — chlorotic tissue). The component hue (in degrees) was then used to calculate the green and the greener fractions as the sum of green pixels included in the hue angle ranges of $60^\circ \leq \text{hue} \leq 180^\circ$ and $80^\circ \leq \text{hue} \leq 180^\circ$, respectively. These fractions were used to calculate the senescence index (SI), which indicates the degree of plant senescence, as follows (Genangeli et al., 2023):

$$\text{SI} = (\text{GAS} - \text{GerAS})/\text{GAS} \quad (2)$$

where GAS is the green area calculated from the side view, corresponding to the sum of the pixels in the hue angular region from 60° to 180°. GerAS is the greener area calculated from the side view, corresponding to the sum of the pixels in the hue angular region from 80° to 180°.

NIR images were acquired from a side view. The final results were expressed as NIR intensities obtained at the typical water absorption wavelength of 1,450 nm as previously reported in Kim et al. (2020). An increased level of absorption determines a reduced level of reflectance in the NIR spectrum, corresponding to an increased water content of plant tissues. Vine tissues with a high-water content showed a low NIR intensity.

5.2.6 Data analysis

Statistical analysis was performed using the R software (4.3.3 version, R foundation for Statistical Computing, Vienna, Austria), and data were plotted using Sigmaplot 15.0 (Systat Software, Inc., San Jose, CA, USA). Both physiological and phenotyping measurements were reported as mean values and standard error of the means ($\pm$ SE). The normal distribution of the data was evaluated both visually (QQ-plot) and statistically (Shapiro-Wilk normality test). Levene's test was used to check for homogeneity of variances. Two-way ANOVA analysis was used to determine the effects of irrigation treatments and cultivars on physiological and phenotypic parameters at each DAW. One-way ANOVA was used to examine the differences between irrigation treatments within each cultivar at each DAW. Multiple comparisons of means between irrigation treatments and cultivars

within each DAW were made using Tukey's honest significant difference (HSD) *post-hoc* test with *p*-values <0.05 considered significant.

5.3 Results

5.3.1 Effect of waterlogging on the photosynthetic capacity

The WW vines showed a similar behavior for both the D and H rootstocks, with values of *g_s* approximately 0.1-0.14 mol m⁻² s⁻¹ and *E* approximately 2.3-3.5 mmol m⁻² s⁻¹. The B rootstock was characterized by slightly higher values, peaking at 0.2 mol m⁻² s⁻¹ and 4.6 mmol m⁻² s⁻¹, respectively. A significant effect of cultivar was observed throughout the experiment for *g_s* and *E*, with a lesser treatment effect appearing from DAW 5 and 4, respectively. WL vines of the D and H rootstocks showed a significant and progressive decrease in *g_s* and *E* from DAW 4. Instead, vines of the B rootstock subjected to waterlogging showed *g_s* and *E* values similar to those of WW vines, with no significant differences on all days of stress (Figure 1). In particular, significant reductions of approximately 80%-85% in *g_s* and 70%-75% in *E* were observed in WL vines of the D and H rootstocks at the end of the waterlogging stress experiment (DAW 9) (Figures 1A, B).

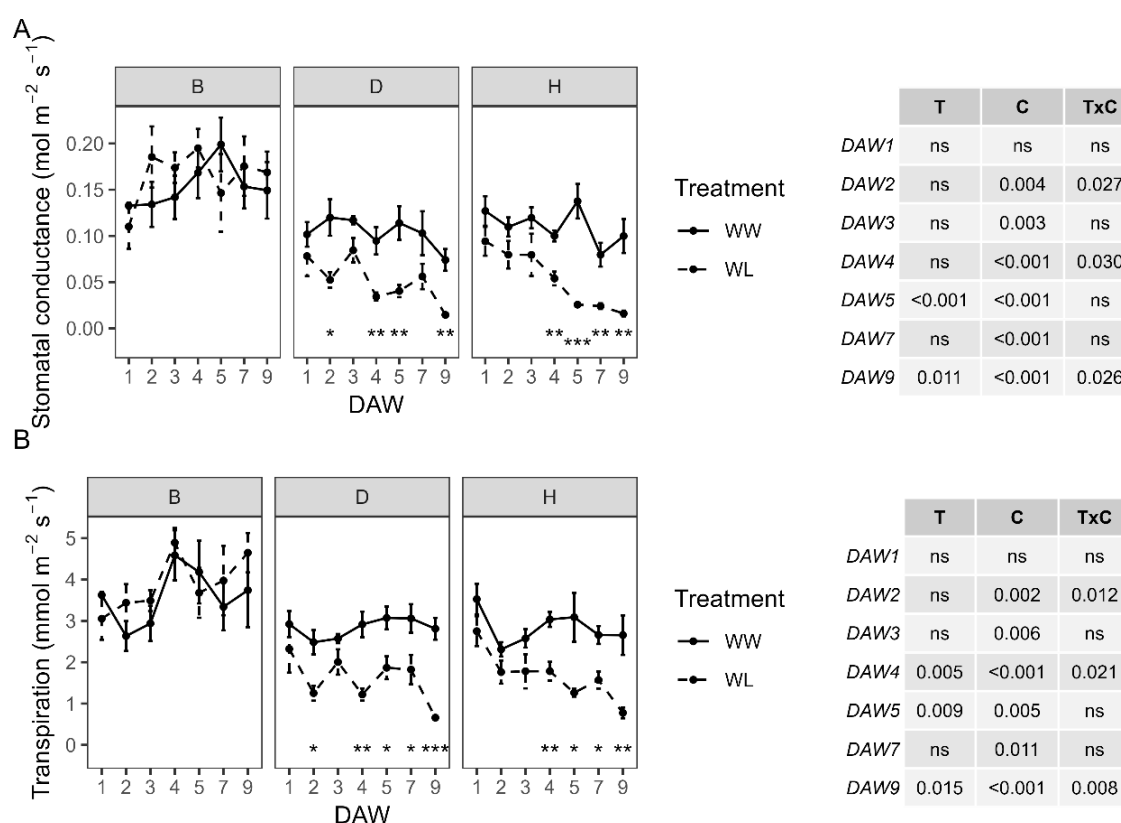


Figure 1 Effects of waterlogging stress on (A) stomatal conductance (*g_s*) and (B) transpiration (*E*) of different rootstocks (B, D, H) during waterlogging days (11:00 h). Two-way ANOVA *p*-values are shown in the tables for both *g_s* and *E* and for each DAW. Asterisks denote significant

differences according to one-way ANOVA between WW and WL for each parameter and rootstock. Data shown are means $\pm$ standard error of the means ($n = 4$). T, treatment effects; C, cultivar effects; ns, not significant at $p \geq 0.05$.

Z/D and Z/H combinations showed a similar behavior in g_s and E , both under well-watered and stressed conditions. The Z/B combination was characterized by slightly higher values in WW vines compared to the other combinations and a slower decline in physiological parameters in WL vines compared to WW vines (Figure 2). A consistently significant ($p < 0.001$) effect of the waterlogging treatment was observed throughout the experiment from the start of stress application for both physiological indicators. Minimum g_s values, less than $0.05 \text{ mol m}^{-2} \text{ s}^{-1}$, were reached in WL vines of all combinations at the end of the waterlogging stress experiment (DAW 9). In the Z/B combination, g_s of WL vines remained within the range of control values from DAW 1 to DAW 4, then started to decrease significantly at DAW 5. A minimum of $0.03 \text{ mol m}^{-2} \text{ s}^{-1}$ was reached in the Z/B combination after 9 days of waterlogging stress, with a reduction of approximately 86% compared to WW vines. For the Z/D and Z/H combinations, WL vines already had g_s values below $0.05 \text{ mol m}^{-2} \text{ s}^{-1}$ from DAW 2 and DAW 5, respectively. Reductions in g_s of approximately 93% and 87% compared to WW vines were achieved in Z/D and Z/H combinations, respectively, at the end of the waterlogging stress experiment (Figure 2A). Minimum E values of less than $1 \text{ mmol m}^{-2} \text{ s}^{-1}$ were reached in WL vines of the Z/D and Z/H combinations between DAW 7 and DAW 9, while minimum values of approximately $2 \text{ mmol m}^{-2} \text{ s}^{-1}$ were reached in the Z/B combination from DAW 5. At the end of the experiment, E was reduced by approximately 51%, 78%, and 65% in the Z/B, Z/D, and Z/H combinations, respectively (Figure 2B).

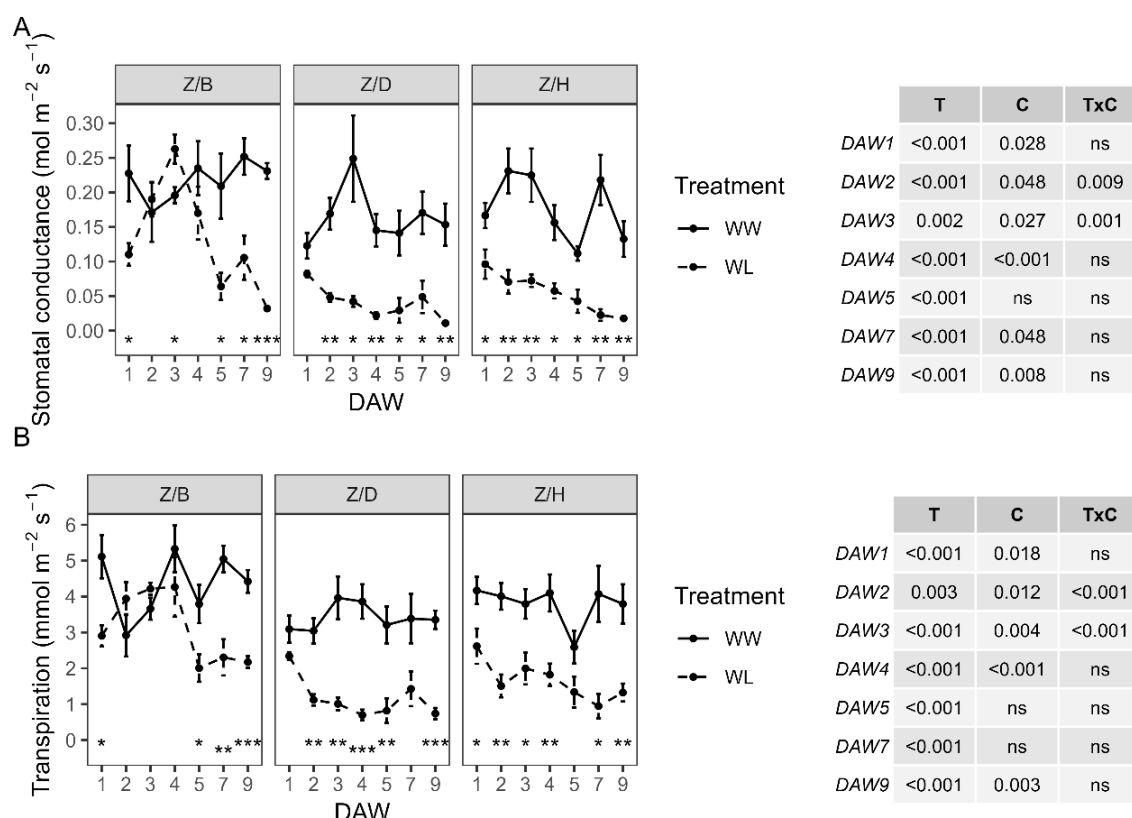


Figure 2 Effects of waterlogging stress on (A) stomatal conductance (g_s) and (B) transpiration (E) of different scion-rootstock combinations (Z/B, Z/D, Z/H) during waterlogging days (10:00 h). Two-way ANOVA p -values are shown in the tables for both g_s and E and for each DAW. Asterisks denote significant differences according to one-way ANOVA between WW and WL for each parameter and scion-rootstock combination. Data shown are means $\pm$ standard error of the means ($n = 4$). T, treatment effects; C, cultivar effects; ns, not significant at $p \geq 0.05$.

Diurnal trends of g_s , E , and A measured 3 days after waterlogging stress are shown in Figure 3 to highlight the physiological behavior of the WW and WL vines of rootstocks and scion-rootstock combinations throughout the day. The physiological activity of the WW rootstocks appeared to be less intense than that of the combinations, with slightly lower values for g_s , E , and A . In addition, the rootstocks appeared to be more resilient to waterlogging stress than the combinations, which showed a more pronounced decrease in physiological parameters under waterlogging stress. Stomatal conductance was higher in the morning hours and started to decrease from the early afternoon in WW vines of all rootstocks (Figure 3A) and Z/D and Z/H combinations (Figure 3D). In the Z/B combination, g_s reached higher values in the early afternoon (Figure 3D). The WL vines of the D and H rootstocks and the Z/D and Z/H combinations showed an almost constant g_s throughout the day, reaching values greatly reduced compared to the WW vines. On the other

hand, the WL vines of the B rootstock and the Z/B combination behaved similarly to the WW vines, and only a slight reduction in g_s was observed in the afternoon hours for the Z/B combination (Figures 3A, D). The E of the WW vines followed the g_s , increasing slightly in the middle hours of the morning, when light and other environmental parameters become more demanding, and then starting to decrease from the early afternoon. The WL vines of the Z/D combination had the lowest E values, which were almost constant throughout the day (Figure 3E). The A showed a similar behavior to the g_s and was strongly affected by waterlogging stress (Figures 3C, F). In particular, greater reductions in A were observed in WL vines of the D and H rootstocks (Figure 3C) and the Z/D and Z/H combinations (Figure 3F), while WL vines of the B rootstock and the Z/B combination maintained values similar to those of WW vines.

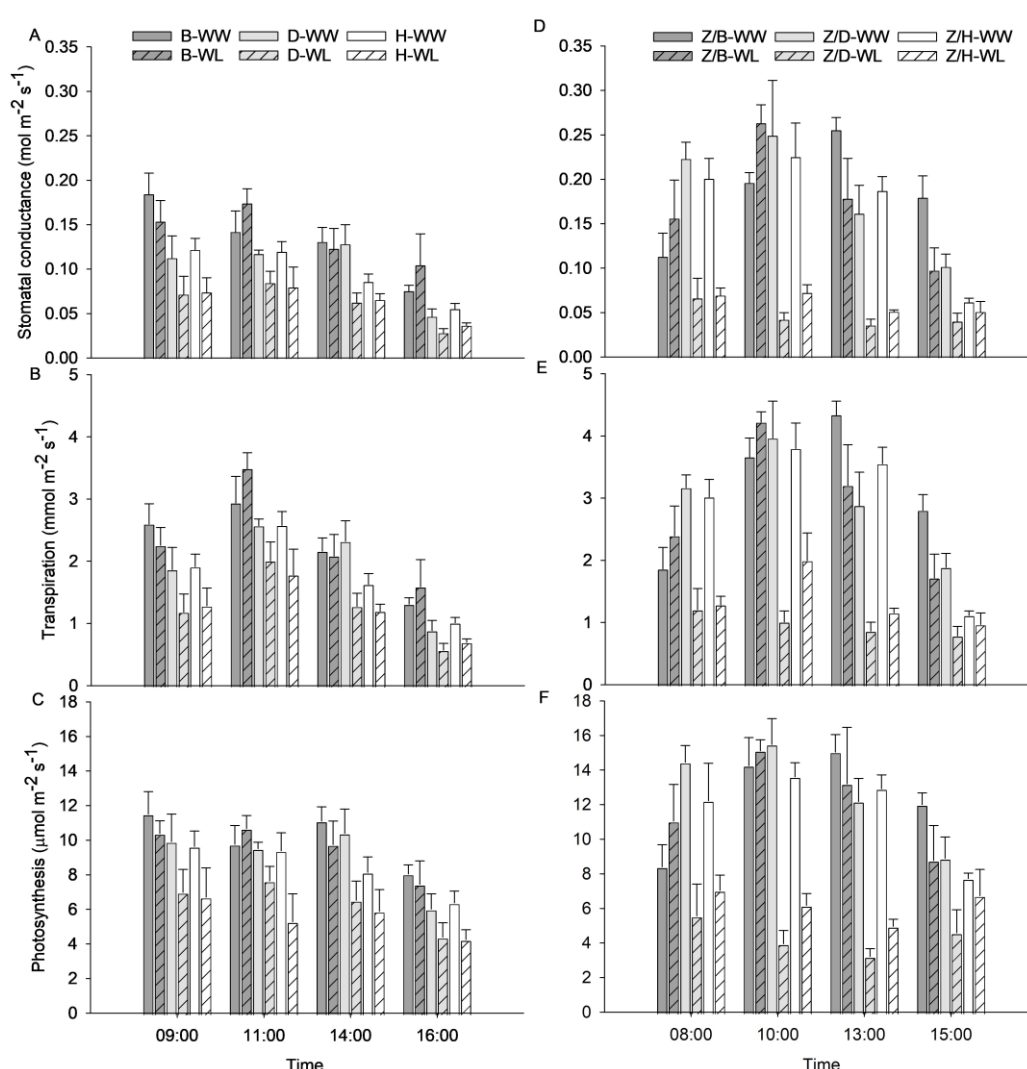


Figure 3 Diurnal trends of (A, D) stomatal conductance (g_s), (B, E) transpiration (E), and (C, F) photosynthesis (A) of the different rootstocks (B, D, H) and scion-rootstock combinations (Z/B,

Z/D, Z/H) 3 days after waterlogging stress. Data presented are means $\pm$ standard error of the means ($n = 4$).

The D and H rootstocks had similar A values, while slightly higher values were generally observed in the B rootstock, with significant differences between rootstocks at DAW 2. As reported in Table 1, A of the D and H rootstocks gradually decreased with prolonged waterlogging exposure. A significant reduction in A due to waterlogging stress was observed in the D and H rootstocks at DAW 4, with values reduced by approximately 63% and 47%, respectively, compared to WW rootstocks. The B rootstock was unaffected by waterlogging, with values similar to those of the WW rootstocks (Table 1). All WL rootstocks showed no changes in C_i values during the first 4 days of waterlogging stress, suggesting that the stress had not yet affected the balance between CO_2 intake (controlled by stomatal conductance) and CO_2 fixation (controlled by photosynthetic capacity). However, significant differences in C_i were observed between B and D and H rootstocks at DAW 3 and DAW 4, with the B rootstock showing slightly higher values, which may be associated with the higher g_s (Figure 1A) and then CO_2 intake (Table 1). The WUE_i was not affected by waterlogging stress during the first 4 days of the experiment for any of the rootstocks. Lower WUE_i values were observed for the B rootstock compared to the D and H rootstocks throughout the 4 days, with significant differences at DAW 3 and DAW 4, suggesting a lower water use efficiency for the B rootstock (Table 1). In particular, high g_s values in the B rootstock resulted in increased CO_2 intake through the open stomata, but at the same time, more water was lost through transpiration. Higher values of C_i indicated the inability of the photosynthetic apparatus to use the increased CO_2 effectively, leading to an accumulation of CO_2 in the leaf, and lower values of WUE_i indicated that the vines used more water (high g_s , E) to assimilate the same amount of carbon. A significant increase in T_{leaf} due to waterlogging stress was observed at DAW 4, with values increased by approximately 2% and 1% in WL vines of D and H rootstocks, respectively, compared to WW rootstocks. The T_{leaf} of the B rootstock was not affected by waterlogging and showed values in the range of the WW rootstock (Table 1). Lower values of T_{leaf} were generally observed for the B rootstock, followed by the H and D rootstocks, suggesting that higher g_s and E increased the cooling effect of the leaves in the B rootstock (Figures 1A, B).

Table 1 Effects of waterlogging stress on photosynthesis (A), internal CO₂ concentration (C_i), intrinsic water use efficiency (WUE_i), and leaf temperature (T_{leaf}) in different rootstocks (B, D, H) during the first 4 days of the experiment (11:00 h). The means of each rootstock are compared within the same row, the means of each irrigation treatment are compared within the same column, and the interaction between all values is assessed using Tukey's honest significant difference (HSD) comparison test ($p < 0.05$). Different lowercase letters indicate significant differences in the means of each treatment and each rootstock, while different uppercase letters indicate significant differences of the interaction ($n = 4 \pm \text{SE}$). Note that letters were not reported when there were no significant differences.

	Treatment	Rootstock			Treatment	Rootstock			Treatment
		B	D	H		B	D	H	
		A ($\mu\text{mol m}^{-2} \text{s}^{-1}$)				WUEi ($\mu\text{mol CO}_2 \text{mol}^{-1} \text{H}_2\text{O}$)			
DAW 1	Well-watered	10.55 $\pm$ 0.34	8.46 $\pm$ 0.56	10.09 $\pm$ 1.19	9.70 $\pm$ 0.49	79.44 $\pm$ 2.06	85.69 $\pm$ 6.39	81.04 $\pm$ 7.90	82.06 $\pm$ 3.23
	Waterlogged	8.01 $\pm$ 1.45	7.03 $\pm$ 1.72	8.43 $\pm$ 1.31	7.82 $\pm$ 0.80	74.43 $\pm$ 3.05	92.93 $\pm$ 3.86	90.58 $\pm$ 5.04	85.98 $\pm$ 3.26
	Rootstock	9.28 $\pm$ 0.84	7.75 $\pm$ 0.88	9.26 $\pm$ 0.88	-	76.94 $\pm$ 1.95	89.31 $\pm$ 3.72	85.81 $\pm$ 4.70	-
DAW 2	Well-watered	9.73 $\pm$ 1.03 ^{AB}	9.47 $\pm$ 1.23 ^{AB}	7.96 $\pm$ 0.73 ^{AB}	9.05 $\pm$ 0.58	75.69 $\pm$ 6.54	80.46 $\pm$ 3.69	74.02 $\pm$ 7.51	76.72 $\pm$ 3.31
	Waterlogged	11.44 $\pm$ 1.78 ^A	4.65 $\pm$ 0.89 ^B	6.14 $\pm$ 1.37 ^{AB}	7.41 $\pm$ 1.14	63.03 $\pm$ 3.56	86.82 $\pm$ 7.61	74.74 $\pm$ 6.81	74.86 $\pm$ 4.38
	Rootstock	10.58 $\pm$ 1.01 ^a	7.06 $\pm$ 1.15 ^b	7.05 $\pm$ 0.80 ^b	-	69.36 $\pm$ 4.19	83.64 $\pm$ 4.10	74.38 $\pm$ 4.70	-
DAW 3	Well-watered	9.76 $\pm$ 1.10	9.51 $\pm$ 0.38	9.38 $\pm$ 1.04	9.55 $\pm$ 0.47	72.12 $\pm$ 7.40	81.34 $\pm$ 2.74	78.43 $\pm$ 4.39	77.30 $\pm$ 2.96
	Waterlogged	10.67 $\pm$ 0.76	7.64 $\pm$ 0.84	5.29 $\pm$ 1.60	7.87 $\pm$ 0.89	61.81 $\pm$ 1.64	93.46 $\pm$ 8.33	66.10 $\pm$ 6.15	73.79 $\pm$ 5.28
	Rootstock	10.21 $\pm$ 0.64	8.58 $\pm$ 0.55	7.34 $\pm$ 1.18	-	66.97 $\pm$ 4.01 ^b	87.40 $\pm$ 4.66 ^a	72.26 $\pm$ 4.20 ^{ab}	-
DAW 4	Well-watered	11.43 $\pm$ 1.26 ^A	8.58 $\pm$ 1.12 ^{AB}	8.48 $\pm$ 0.40 ^{AB}	9.50 $\pm$ 0.67 ^a	70.84 $\pm$ 6.87	91.73 $\pm$ 3.97	85.18 $\pm$ 3.42	82.58 $\pm$ 3.70
	Waterlogged	11.78 $\pm$ 1.16 ^A	3.16 $\pm$ 0.59 ^C	4.48 $\pm$ 0.85 ^{BC}	6.47 $\pm$ 1.24 ^b	70.00 $\pm$ 3.57	89.24 $\pm$ 7.97	82.52 $\pm$ 6.87	77.59 $\pm$ 4.94
	Rootstock	11.61 $\pm$ 0.79 ^a	5.87 $\pm$ 1.18 ^b	6.48 $\pm$ 0.87 ^b	-	65.92 $\pm$ 4.04 ^b	90.49 $\pm$ 4.15 ^a	83.85 $\pm$ 3.59 ^a	-
		Ci ($\mu\text{mol mol}^{-1}$)				T_{leaf} (°C)			
DAW 1	Well-watered	260.92 $\pm$ 3.56	253.60 $\pm$ 9.44	258.74 $\pm$ 13.26	257.76 $\pm$ 5.11	32.45 $\pm$ 0.03	32.88 $\pm$ 0.13	32.59 $\pm$ 0.07	32.64 $\pm$ 0.07
	Waterlogged	271.91 $\pm$ 3.75	243.81 $\pm$ 4.20	245.61 $\pm$ 8.14	253.78 $\pm$ 4.89	32.61 $\pm$ 0.21	33.03 $\pm$ 0.11	32.91 $\pm$ 0.18	32.85 $\pm$ 0.10
	Rootstock	266.42 $\pm$ 3.17	248.71 $\pm$ 5.13	252.18 $\pm$ 7.62	-	32.53 $\pm$ 0.10 ^b	32.95 $\pm$ 0.08 ^a	32.75 $\pm$ 0.11 ^{ab}	-
DAW 2	Well-watered	272.33 $\pm$ 9.54	264.71 $\pm$ 5.02	277.02 $\pm$ 12.44	271.35 $\pm$ 5.19	32.34 $\pm$ 0.09	32.59 $\pm$ 0.17	32.48 $\pm$ 0.18	32.47 $\pm$ 0.08
	Waterlogged	289.96 $\pm$ 4.96	260.60 $\pm$ 13.06	278.00 $\pm$ 12.05	276.19 $\pm$ 6.64	32.24 $\pm$ 0.18	33.07 $\pm$ 0.16	32.73 $\pm$ 0.21	32.68 $\pm$ 0.14
	Rootstock	281.15 $\pm$ 5.99	262.66 $\pm$ 6.52	277.51 $\pm$ 8.02	-	32.29 $\pm$ 0.10 ^b	32.83 $\pm$ 0.14 ^a	32.61 $\pm$ 0.14 ^{ab}	-
DAW 3	Well-watered	277.25 $\pm$ 10.72	262.75 $\pm$ 4.66	267.71 $\pm$ 7.82	269.23 $\pm$ 4.61	32.50 $\pm$ 0.09	32.65 $\pm$ 0.21	32.47 $\pm$ 0.09	32.54 $\pm$ 0.08
	Waterlogged	292.33 $\pm$ 1.83	245.65 $\pm$ 12.69	292.35 $\pm$ 10.33	276.78 $\pm$ 8.29	32.41 $\pm$ 0.05	32.93 $\pm$ 0.04	32.41 $\pm$ 0.24	32.58 $\pm$ 0.10
	Rootstock	284.79 $\pm$ 5.79 ^a	254.20 $\pm$ 7.04 ^b	280.03 $\pm$ 7.59 ^a	-	32.45 $\pm$ 0.05	32.79 $\pm$ 0.11	32.44 $\pm$ 0.12	-
DAW 4	Well-watered	272.39 $\pm$ 9.82	242.75 $\pm$ 6.09	253.49 $\pm$ 5.45	256.21 $\pm$ 5.34	33.06 $\pm$ 0.12	33.74 $\pm$ 0.19	33.46 $\pm$ 0.08	33.42 $\pm$ 0.11 ^b
	Waterlogged	288.45 $\pm$ 6.15	252.79 $\pm$ 13.08	262.44 $\pm$ 11.35	267.89 $\pm$ 7.16	32.98 $\pm$ 0.07	34.31 $\pm$ 0.08	33.77 $\pm$ 0.17	33.69 $\pm$ 0.17 ^a
	Rootstock	280.42 $\pm$ 6.16 ^a	247.77 $\pm$ 6.94 ^b	257.97 $\pm$ 6.07 ^b	-	33.02 $\pm$ 0.06 ^c	34.03 $\pm$ 0.14 ^a	33.61 $\pm$ 0.11 ^b	-

Waterlogging caused significant changes in A of the scion-rootstock combinations during all the first 4 days of the experiment. In particular, the reductions in A were more pronounced in the Z/D and Z/H combinations, where A decreased from DAW 1, and with prolonged waterlogging exposure, by approximately 89% and 52%, respectively, at DAW 4 (Table 2). Higher values of A were generally observed in the Z/B combination, with significant differences at DAW 3 and DAW 4, reinforced by the little or no effect of waterlogging on the Z/B combination compared to Z/D and Z/H combinations. Slight reductions in A were observed in the Z/B combination only at DAW 1, consistent with the response in g_s and E (Figures 2A, B), and at DAW 4, but with no significant differences. The C_i values were significantly lower in the WL treatment at DAW 1, consistent with the reductions in g_s and E observed in the combinations (Figures 2A, B). This suggests that lower g_s affected CO_2 intake, leading to a decrease in C_i , which in turn limited photosynthetic activity due to less CO_2 available for fixation (Table 2). This tendency of C_i is maintained in the Z/D and Z/H combinations until DAW 3 and DAW 4, respectively, supporting the occurrence of moderate water stress during which the WL vines initially showed a decrease in C_i due to partial stomatal closure (Figure 2A). After 4 days of waterlogging stress, an inverse trend for C_i was observed in the WL vines of the Z/D combination, which showed significantly higher values compared to the WW vines. At this time, a more severe water stress condition occurred, as indicated by the rapid decrease in A (equal to $1.30 \mu\text{mol m}^{-2} \text{s}^{-1}$) and the strong impairment of the photosynthetic apparatus, which was no longer able to fix CO_2 , leading to an increased C_i concentration. The Z/B combination showed no changes in C_i values for the remaining days (DAW 2-4), suggesting that the stress had not yet affected the balance between CO_2 intake and CO_2 fixation, as supported by the still control values of g_s and A (Figure 2A; Table 2). Different from the rootstocks, the WUE_i of the rootstock-scion combinations was affected by waterlogging stress. Higher values were observed in the Z/D and Z/H combinations under waterlogging until DAW 3 and DAW 4, respectively, suggesting a more efficient use of water from water lost through transpiration (low g_s) and carbon assimilation. An inverse trend, as for C_i , was also found for WUE_i 4 days after waterlogging stress in the Z/D combination, which decreased, confirming the severe impairment of the photosynthetic apparatus (Table 2). For the Z/B combination, higher WUE_i values were observed in the WL vines compared to the WW vines on DAW 1 and DAW 4, confirming the observations related to other parameters on these days and suggesting that DAW 4 represented the beginning of the stress response for the Z/B combination, although no significant differences were already observed. Higher T_{leaf} was found in the WL treatment compared to the WW treatment on all 4 days of waterlogging stress. In particular, T_{leaf} increased progressively in Z/D and Z/H combinations under waterlogging from DAW 2, reaching increases of approximately 4% and 2%, respectively, compared to the WW

vines at DAW 4. The Z/B combination generally showed lower values of T_{leaf} compared to other combinations, and WL vines maintained values similar to those of the WW vines during the first 4 days of waterlogging stress (Table 2).

Table 2 Effects of waterlogging stress on photosynthesis (A), internal CO₂ concentration (Ci), intrinsic water use efficiency (WUEi), and leaf temperature (T_{leaf}) in different scion-rootstock combinations (Z/B, Z/D, Z/H) during the first 4 days of waterlogging (10:00 h). The means of each scion-rootstock combination are compared within the same row, the means of each irrigation treatment are compared within the same column, and the interaction between all values is assessed using Tukey's honest significant difference (HSD) comparison test ($p < 0.05$). Different lowercase letters indicate significant differences in the means of each treatment and each scion-rootstock combination, while different uppercase letters indicate significant differences of the interaction ($n = 4 \pm \text{SE}$). Note that letters were not reported when there were no significant differences.

	Treatment	Scion-rootstock combination			Treatment	Scion-rootstock combination			Treatment
		Z/B	Z/D	Z/H		Z/B	Z/D	Z/H	
		A ($\mu\text{mol m}^{-2} \text{s}^{-1}$)				WUEi ($\mu\text{mol CO}_2 \text{mol}^{-1} \text{H}_2\text{O}$)			
DAW 1	Well-watered	13.63 $\pm$ 1.43	10.05 $\pm$ 1.52	12.58 $\pm$ 1.10	12.09 $\pm$ 0.84 ^a	62.28 $\pm$ 4.79	81.86 $\pm$ 2.20	76.50 $\pm$ 4.66	73.55 $\pm$ 3.27 ^b
	Waterlogged	8.25 $\pm$ 0.72	7.72 $\pm$ 0.71	8.63 $\pm$ 1.49	8.20 $\pm$ 0.56 ^b	77.40 $\pm$ 6.11	93.68 $\pm$ 3.48	92.76 $\pm$ 4.62	87.95 $\pm$ 3.39 ^a
	Combination	10.94 $\pm$ 1.26	8.89 $\pm$ 0.89	10.61 $\pm$ 1.14	-	69.84 $\pm$ 4.59 ^b	87.77 $\pm$ 2.93 ^a	84.63 $\pm$ 4.32 ^a	-
DAW 2	Well-watered	12.79 $\pm$ 1.46 ^{AB}	13.41 $\pm$ 1.55 ^A	14.69 $\pm$ 1.28 ^A	13.63 $\pm$ 0.79 ^a	90.81 $\pm$ 24.91	80.51 $\pm$ 4.45	65.96 $\pm$ 6.12	79.09 $\pm$ 8.43
	Waterlogged	11.61 $\pm$ 1.09 ^{AB}	5.32 $\pm$ 0.94 ^C	7.46 $\pm$ 1.25 ^{BC}	8.13 $\pm$ 0.98 ^b	62.06 $\pm$ 3.84	109.06 $\pm$ 7.69	113.08 $\pm$ 14.62	94.73 $\pm$ 8.65
	Combination	12.20 $\pm$ 0.87	9.36 $\pm$ 1.75	11.07 $\pm$ 1.60	-	76.44 $\pm$ 12.87	94.78 $\pm$ 6.78	89.52 $\pm$ 11.54	-
DAW 3	Well-watered	14.25 $\pm$ 1.62 ^A	15.48 $\pm$ 1.50 ^A	13.61 $\pm$ 0.82 ^A	14.45 $\pm$ 0.75 ^a	60.52 $\pm$ 3.04	68.06 $\pm$ 7.85	64.10 $\pm$ 7.53	64.23 $\pm$ 3.53 ^b
	Waterlogged	15.12 $\pm$ 0.64 ^A	3.96 $\pm$ 0.76 ^B	6.17 $\pm$ 0.69 ^B	8.42 $\pm$ 1.50 ^b	58.22 $\pm$ 3.29	95.65 $\pm$ 8.56	87.95 $\pm$ 11.03	80.60 $\pm$ 6.51 ^a
	Combination	14.68 $\pm$ 0.82 ^a	9.72 $\pm$ 2.31 ^b	9.89 $\pm$ 1.49 ^b	-	59.37 $\pm$ 2.12 ^b	81.85 $\pm$ 7.49 ^a	76.02 $\pm$ 7.65 ^{ab}	-
DAW 4	Well-watered	14.04 $\pm$ 1.60 ^A	11.62 $\pm$ 0.94 ^{AB}	11.99 $\pm$ 1.36 ^{AB}	12.55 $\pm$ 0.76 ^a	62.29 $\pm$ 5.90 ^B	82.94 $\pm$ 5.88 ^{AB}	79.21 $\pm$ 5.87 ^{AB}	74.81 $\pm$ 4.10
	Waterlogged	11.95 $\pm$ 2.30 ^{AB}	1.30 $\pm$ 0.64 ^C	5.71 $\pm$ 0.85 ^{BC}	6.32 $\pm$ 1.52 ^b	74.60 $\pm$ 7.48 ^{AB}	50.23 $\pm$ 14.68 ^B	102.00 $\pm$ 7.66 ^A	75.61 $\pm$ 8.41
	Combination	13.00 $\pm$ 1.35 ^a	6.46 $\pm$ 2.02 ^b	8.85 $\pm$ 1.40 ^b	-	68.45 $\pm$ 4.98 ^b	66.58 $\pm$ 9.58 ^b	90.61 $\pm$ 6.21 ^a	-
		Ci ($\mu\text{mol mol}^{-1}$)				T_{leaf} (°C)			
DAW 1	Well-watered	284.96 $\pm$ 6.14	259.02 $\pm$ 4.54	263.45 $\pm$ 7.92	269.15 $\pm$ 4.76 ^a	32.10 $\pm$ 0.27	32.29 $\pm$ 0.13	32.39 $\pm$ 0.14	32.26 $\pm$ 0.11 ^b
	Waterlogged	267.84 $\pm$ 9.27	241.47 $\pm$ 6.39	243.04 $\pm$ 5.59	250.78 $\pm$ 5.26 ^b	32.44 $\pm$ 0.25	32.79 $\pm$ 0.09	32.52 $\pm$ 0.14	32.58 $\pm$ 0.10 ^a
	Combination	276.40 $\pm$ 6.08 ^a	250.25 $\pm$ 4.92 ^b	253.25 $\pm$ 5.92 ^b	-	32.27 $\pm$ 0.18	32.54 $\pm$ 0.12	32.45 $\pm$ 0.09	-
DAW 2	Well-watered	245.23 $\pm$ 38.35	260.34 $\pm$ 6.63	281.11 $\pm$ 9.18	262.23 $\pm$ 12.85	31.66 $\pm$ 0.11 ^B	31.90 $\pm$ 0.07 ^{AB}	31.88 $\pm$ 0.25 ^{AB}	31.81 $\pm$ 0.09 ^b
	Waterlogged	285.92 $\pm$ 7.35	225.25 $\pm$ 13.02	216.29 $\pm$ 22.48	242.49 $\pm$ 12.38	31.30 $\pm$ 0.14 ^B	32.57 $\pm$ 0.20 ^A	32.46 $\pm$ 0.14 ^A	32.11 $\pm$ 0.19 ^a
	Combination	265.58 $\pm$ 19.64	242.80 $\pm$ 9.47	248.70 $\pm$ 16.63	-	31.48 $\pm$ 0.11 ^b	32.24 $\pm$ 0.16 ^a	32.17 $\pm$ 0.17 ^a	-
DAW 3	Well-watered	293.59 $\pm$ 4.27	277.18 $\pm$ 10.75	285.97 $\pm$ 12.12	285.58 $\pm$ 5.44	31.57 $\pm$ 0.16 ^C	31.93 $\pm$ 0.23 ^{BC}	31.87 $\pm$ 0.11 ^{BC}	31.79 $\pm$ 0.10 ^b
	Waterlogged	293.78 $\pm$ 5.13	256.50 $\pm$ 10.89	254.25 $\pm$ 15.28	268.18 $\pm$ 8.02	31.69 $\pm$ 0.08 ^{BC}	33.17 $\pm$ 0.35 ^A	32.56 $\pm$ 0.18 ^{AB}	32.47 $\pm$ 0.22 ^a
	Combination	293.69 $\pm$ 3.09 ^a	266.84 $\pm$ 8.09 ^b	270.11 $\pm$ 10.84 ^{ab}	-	31.63 $\pm$ 0.09 ^b	32.55 $\pm$ 0.30 ^a	32.22 $\pm$ 0.16 ^a	-
DAW 4	Well-watered	284.13 $\pm$ 8.31 ^{AB}	253.97 $\pm$ 8.15 ^B	259.44 $\pm$ 8.19 ^{AB}	265.84 $\pm$ 5.84	32.01 $\pm$ 0.18 ^C	32.43 $\pm$ 0.10 ^{BC}	32.65 $\pm$ 0.16 ^{BC}	32.36 $\pm$ 0.11 ^b
	Waterlogged	266.88 $\pm$ 9.81 ^{AB}	315.88 $\pm$ 23.51 ^A	230.97 $\pm$ 11.83 ^B	271.24 $\pm$ 13.48	32.31 $\pm$ 0.17 ^C	33.82 $\pm$ 0.25 ^A	33.19 $\pm$ 0.18 ^{AB}	33.11 $\pm$ 0.21 ^a
	Combination	275.50 $\pm$ 6.78 ^{ab}	284.92 $\pm$ 16.42 ^a	245.21 $\pm$ 8.56 ^b	-	32.16 $\pm$ 0.13 ^b	33.12 $\pm$ 0.29 ^a	32.92 $\pm$ 0.15 ^a	-

Relationships (linear or quadratic) between A and gs, as well as between E and gs, were found in both WL and WW vines of different rootstocks and scion-rootstock combinations (Figure 4). The results indicated that the reduction in gs could be related to a decrease in E and A activity of the vines, which differed according to the cultivar and treatment. No substantial differences in the relationships between A and gs and between E and gs were observed between WW and WL vines of the B rootstock (Figure 4A) and the Z/B combination (Figure 4D), indicating a similar behavior between treatments within the B cultivar. The relationships obtained for the D and H rootstocks showed very similar behavior of these rootstocks to waterlogging stress (Figures 4B, C). The WW vines of the different combinations showed similar relationships between A and gs and between E and gs, whereas the WL vines showed a different behavior (Figures 4D-F).

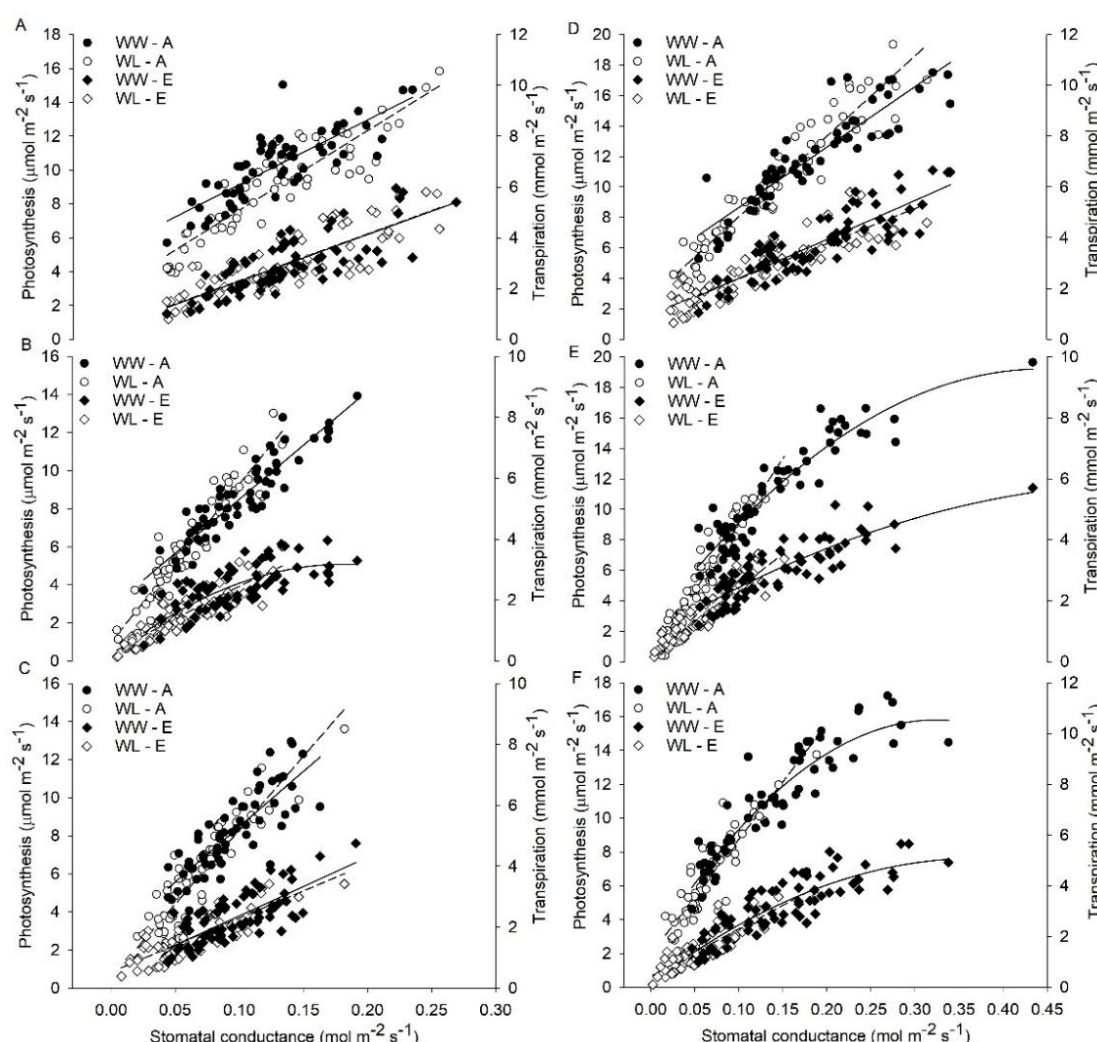


Figure 4 Relationships of stomatal conductance (gs) with photosynthesis (A) and transpiration rate (E) in waterlogged (WL) and well-watered (WW) vines for each rootstock (A) ‘Bounty71’ (B), (B) ‘D1’ (D), (C) ‘Hayward’ (H) and scion-rootstock combination (D) ‘Zesy002/Bounty71’

(Z/B), (E) 'Zesy002/D1' (Z/D), and (F) 'Zesy002/Hayward' (Z/H). Data of all the vines collected during the 9-day waterlogging experiment are shown as individual points.

5.3.2 RGB image-based morphometric and colorimetric parameters

RGB image-based parameters that explain plant morphological and color characteristics, in particular PSA, S, plant color (HUE), and SI, were used to study the response of kiwifruit vines to waterlogging. The WW vines of the B and D rootstocks showed a progressive increase in PSA values over the following days of analysis. PSA differed between cultivars, with D and H showing the highest values approximately 280-335 cm² and 250-270 cm², respectively, and B showing the lowest values approximately 95-140 cm² (Figure 5A). A predominant effect of the cultivar was observed for PSA throughout the experiment, with the treatment effect appearing only from DAW 7. PSA started to differ significantly between WW and WL vines of the D and H rootstocks from DAW 7 and DAW 9, respectively, reaching values reduced by 56% and 32% compared to WW vines (Figure 5A). Projected shoot area and convex hull area from plant images were used to determine leaf density and spread, expressed by the S indicator. S was similar between WW and WL vines of the B and H rootstocks, with no significant differences along the days of waterlogging stress and values approximately 0.3-0.4 and 0.4-0.5, respectively. Instead, it started to differ significantly between WW and WL vines of the D rootstock from DAW 4, resulting in an increase under waterlogging stress. The predominant effect of cultivar was also reported for S and with a lesser effect of treatment (Figure 5B).

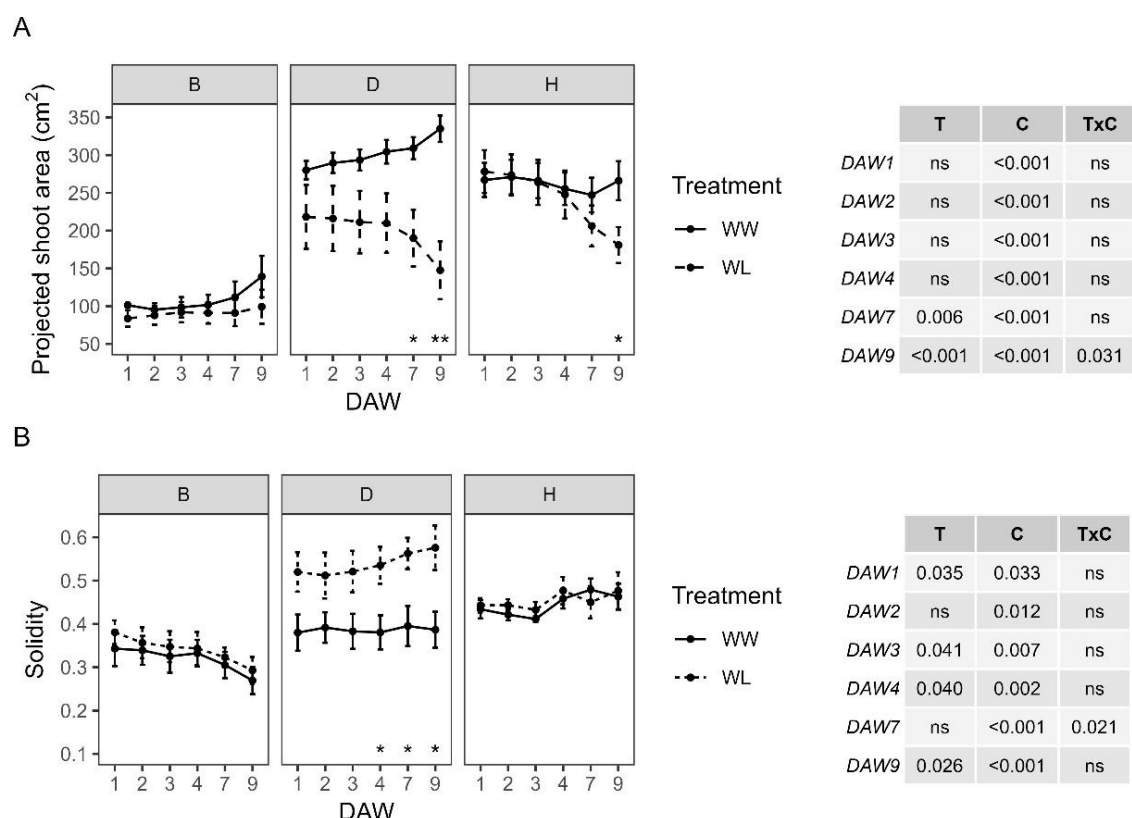


Figure 5 Morphometric parameters derived from the image-based analysis of well-watered (WW) and waterlogged (WL) kiwifruit vines per rootstock (B, D, H): (A) projected shoot area (PSA) and (B) solidity (S). Two-way ANOVA *p*-values are shown in the tables for both PSA and S and for each DAW. Asterisks denote significant differences according to one-way ANOVA between WW and WL for each parameter and rootstock. Data shown are means $\pm$ standard error of the means ($n = 5$). T, treatment effects; C, cultivar effects; ns, not significant at $p \geq 0.05$.

Color changes in kiwifruit vines resulting from the presence of healthy green plant tissue, leaf discoloration, yellowing, or browning of plant tissue were determined by analyzing RGB images with the hue component. During the waterlogging experiment, HUE showed no significant differences between WW and WL vines of each rootstock. However, WL vines of the D and H rootstocks showed a progressive decrease in HUE from DAW 4, whereas those of the B rootstock remained at almost constant values. The WW vines of the D and H rootstocks were similar with values approximately 55°-58°, while those of the B rootstock were slightly higher, above 60° (Figure 6A). The proportion between the green and the greener areas was used to determine the SI. No significant differences were found between WW and WL vines of each rootstock during the days of waterlogging stress (Figure 6B). The B rootstock had the lowest SI compared to the D

and H rootstocks, and the analysis confirmed the prevailing effect of the cultivar for both colorimetric parameters, i.e., HUE and SI, throughout the experiment.

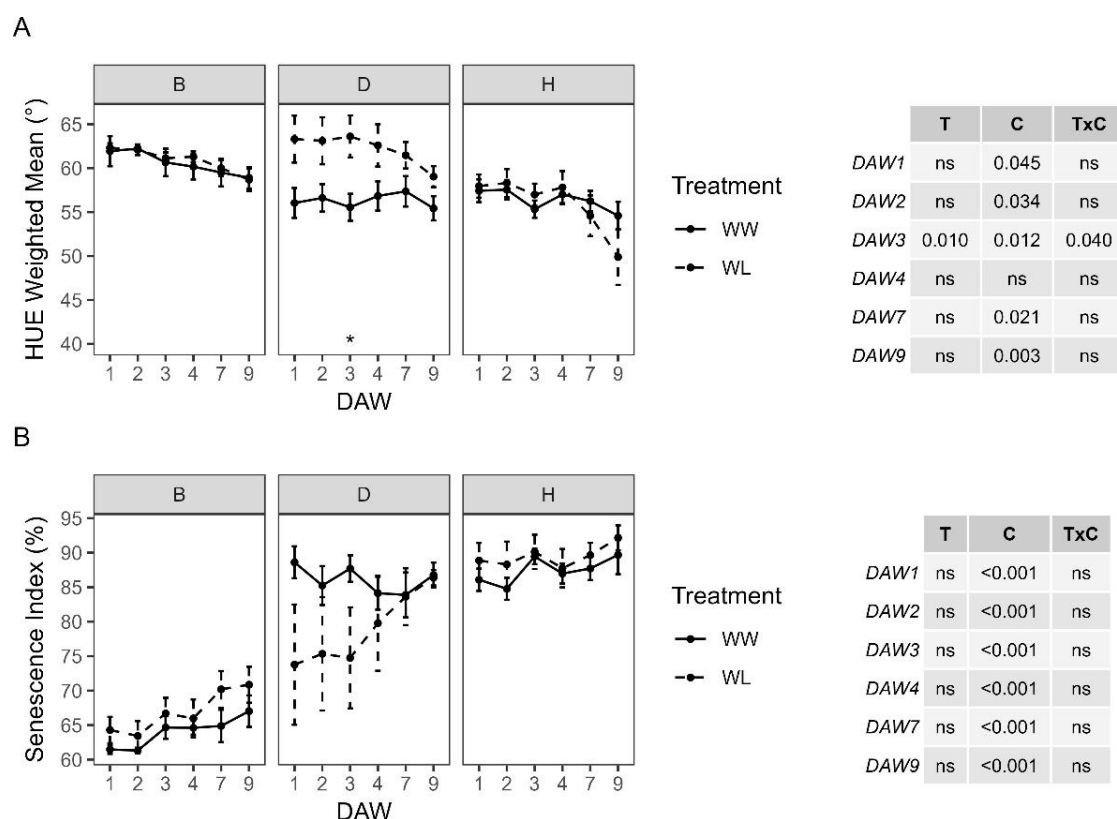


Figure 6 Colorimetric parameters derived from the image-based analysis of well-watered (WW) and waterlogged (WL) kiwifruit vines per rootstock (B, D, H): (A) weighted mean value of the hue channel (HUE) and (B) senescence index (SI). Two-way ANOVA *p*-values are shown in the tables for both HUE and SI and for each DAW. Asterisks denote significant differences according to one-way ANOVA between WW and WL for each parameter and rootstock. Data shown are means $\pm$ standard error of the means ($n = 5$). T, treatment effects; C, cultivar effects; ns, not significant at $p \geq 0.05$.

PSA also varied considerably between the scion-rootstock combinations, with scions grafted on the D and H rootstocks showing the highest values approximately 270-300 cm² and 330-340 cm², respectively, and those grafted on the B rootstock showing the lowest values approximately 210-240 cm² (Figure 7A). A significant effect of the cultivar on PSA was confirmed, with the treatment effect only appearing from DAW 4 and with consistent differences from DAW 7 ($p < 0.001$). Significant differences in PSA between WW and WL vines of the Z/D combination were found from DAW 2, with a decrease of approximately 70% compared to WW vines at the end of the waterlogging experiment. PSA started to decrease in WL vines of the Z/H combination from DAW

4, but with no significant differences compared to WW vines, and remained almost similar between WW and WL vines of the Z/B combination throughout the experiment (Figure 7A). S was similar between WW and WL vines of each scion-rootstock combination, with no significant differences along the days of waterlogging stress and values approximately 0.4-0.5, 0.3-0.5, and 0.45-0.6 for the Z/B, Z/D, and Z/H combinations, respectively (Figure 7B).

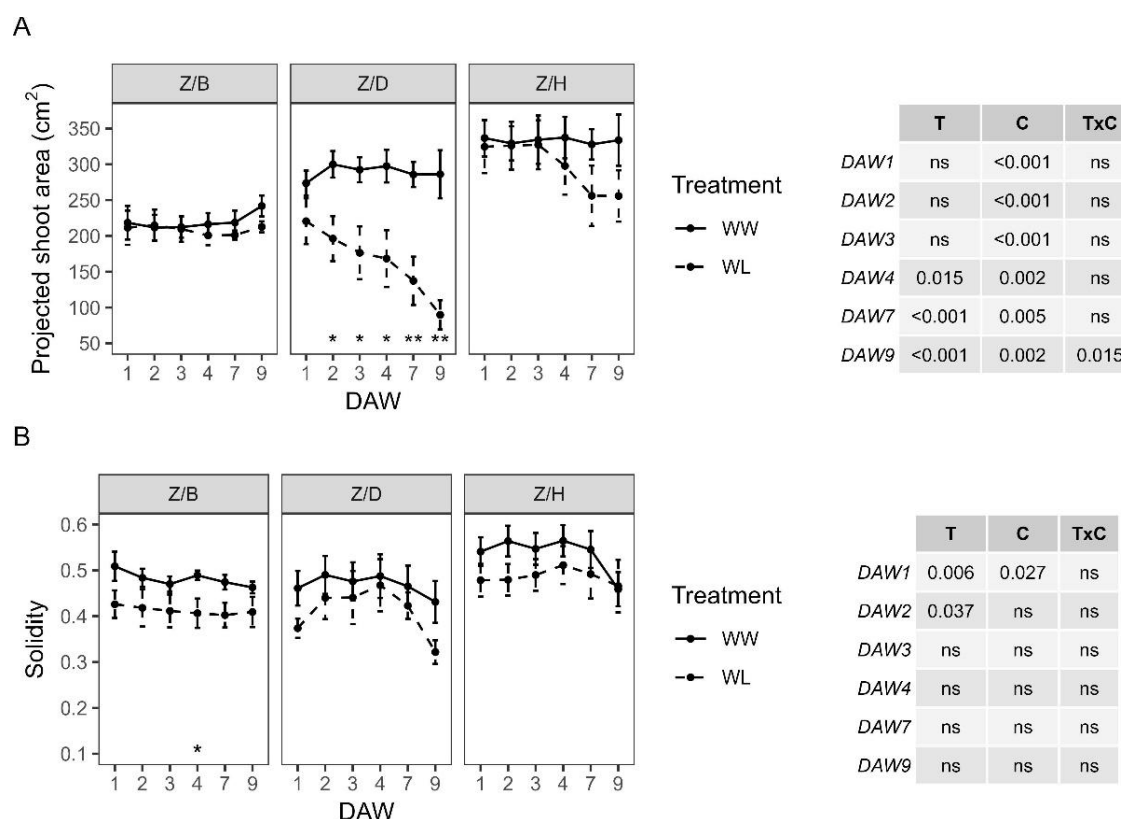


Figure 7 Morphometric parameters derived from the image-based analysis of well-watered (WW) and waterlogged (WL) kiwifruit vines per scion-rootstock combination (Z/B, Z/D, Z/H): (A) projected shoot area (PSA) and (B) solidity (S). Two-way ANOVA *p*-values are shown in the tables for both PSA and S and for each DAW. Asterisks denote significant differences according to one-way ANOVA between WW and WL for each parameter and scion-rootstock combination. Data shown are means $\pm$ standard error of the means ($n = 5$). T, treatment effects; C, cultivar effects; ns, not significant at $p \geq 0.05$.

The HUE of WW vines was almost similar between the different scion-rootstock combinations, showing a slight decrease from DAW 4 in all combinations. No significant differences in HUE were found between WW and WL vines of the Z/B combination. HUE in WL vines of the Z/D and Z/H combinations started to decrease from DAW 4 and differed significantly from WW vines at DAW 9, where both treatment and cultivar had a significant effect (Figure 8A). No significant

effects of cultivar and treatment on SI were found by two-way ANOVA analysis, and no differences were found between WW and WL vines of each scion-rootstock combination during the days of waterlogging stress (Figure 8B).

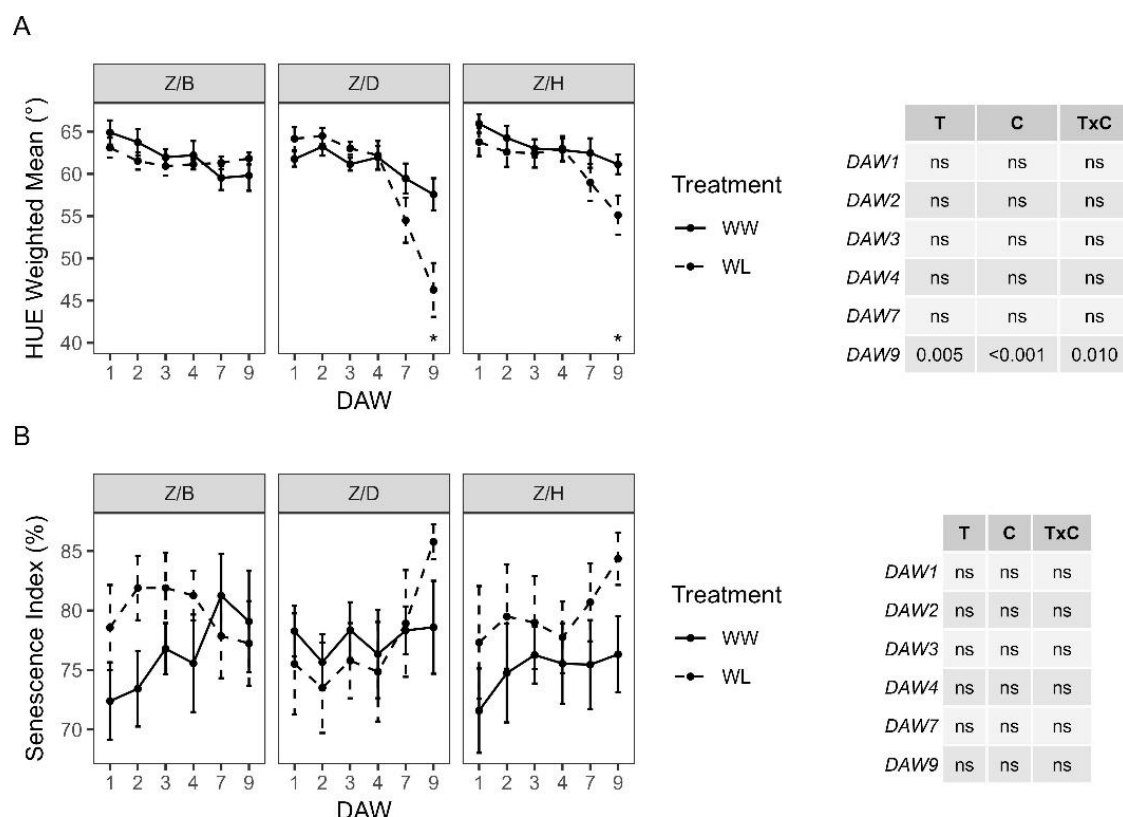


Figure 8 Colorimetric parameters derived from the image-based analysis of well-watered (WW) and waterlogged (WL) kiwifruit vines per scion-rootstock combination (Z/B, Z/D, Z/H): (A) weighted mean value of the hue channel (HUE) and (B) senescence index (SI). Two-way ANOVA *p*-values are shown in the tables for both HUE and SI and for each DAW. Asterisks denote significant differences according to one-way ANOVA between WW and WL for each parameter and scion-rootstock combination. Data shown are means $\pm$ standard error of the means ($n = 5$). T, treatment effects; C, cultivar effects; ns, not significant at $p \geq 0.05$.

5.3.3 Near-infrared imaging

NIR imaging analysis is a common tool used to assess the water content of plants. During the waterlogging days, NIR intensity was similar between WW and WL vines of each rootstock, with no significant differences (Figure 9A). However, regardless of the irrigation treatment applied, the B rootstock showed the highest NIR intensity, with values close to 200, and thus a lower water content in plant tissues compared to the D and H rootstocks, with values between 150 and 170 (Figure 9A). In the scion-rootstock combinations, NIR intensity reached higher values in all WL vines compared to WW vines (Figure 9B). During the last day of waterlogging stress (DAW 9),

NIR intensity differed significantly between WW and WL vines of the Z/D combination, indicating a reduced water content of the vines achieved after the application of 9 days of waterlogging stress (Figure 9B). NIR intensity was then a good indicator of plant stress response, in accordance with the changes in morphometric and colorimetric parameters in response to waterlogging in kiwifruit.

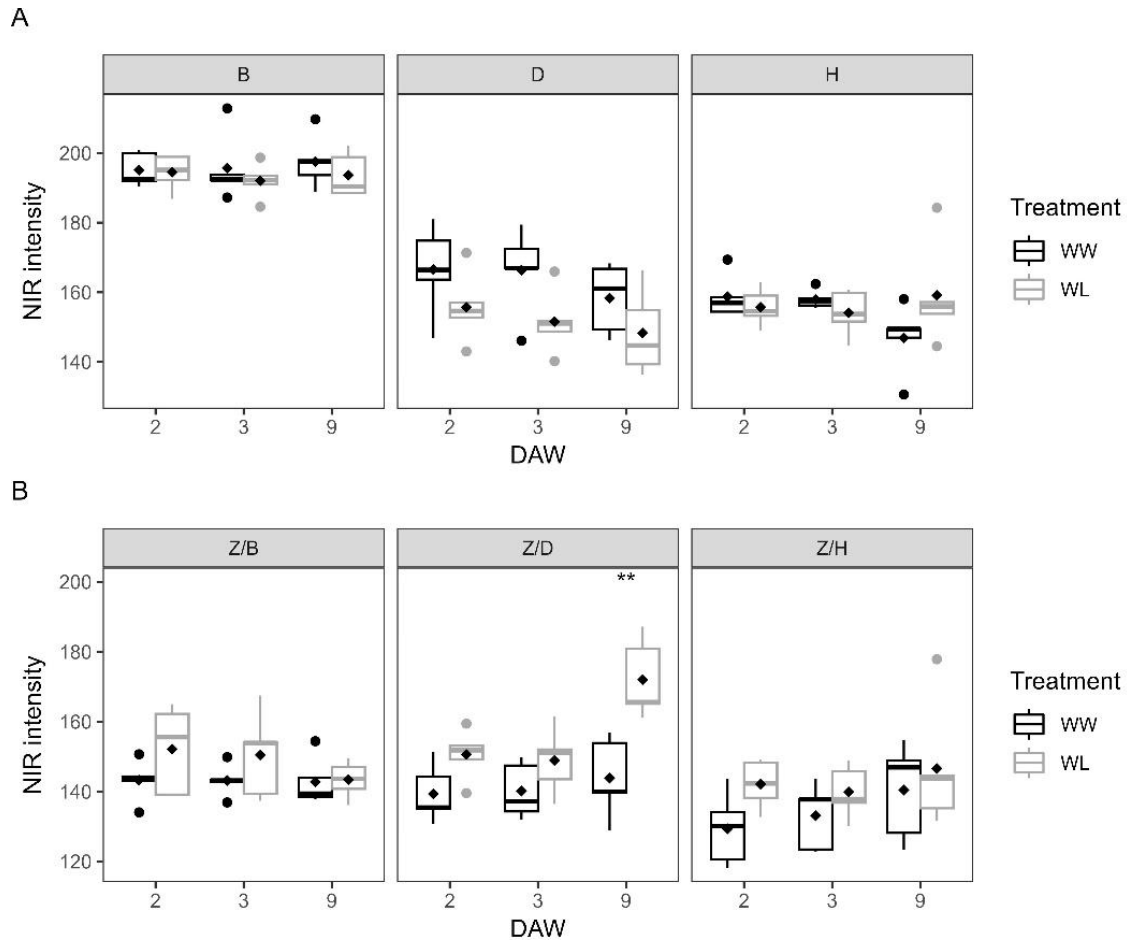


Figure 9 NIR intensities of well-watered (WW) and waterlogged (WL) kiwifruit vines: (A) per rootstock (B, D, H) and (B) per scion-rootstock combination (Z/B, Z/D, Z/H). Asterisks denote significant differences according to one-way ANOVA between WW and WL vines at each DAW. Black diamond symbols represent mean values ($n = 5$).

5.4 Discussion

The results of the present study outlined the differential response of kiwifruit rootstocks and grafting combinations to waterlogging stress. Physiological and phenotypic traits of the vines were combined to increase knowledge of vine physiological behavior and RGB and NIR image-based indicators for assessing waterlogging tolerance/susceptibility in kiwifruit genotypes.

5.4.1 Physiological waterlogging response

Stomatal closure is widely recognized as one of the most rapid responses of many species to waterlogging stress (Kozłowski, 1997; Parent et al., 2008; Xu et al., 2022; Topali et al., 2024). Stomata are responsible for regulating A and E by opening and closing (Kowzłowski, 1997), and their rapid response is correlated with an early reduction in A since the beginning of waterlogging (Xu et al., 2022). Results showed that g_s and therefore E were affected by waterlogging from the first days of the experiment in both D and H rootstocks, but not in the B rootstock. Stomatal closure shortly after waterlogging and a decrease in net photosynthesis have been previously reported in kiwifruit (Li et al., 2020; Li Z. et al., 2021) and in other fruit tree species such as *Prunus* spp (Domingo et al., 2002; Pimentel et al., 2014; Zhang et al., 2023). This tendency was confirmed as the values of A generally decreased with those of g_s , specifically from DAW 4 in the D and H rootstocks and from DAW 2 in the Z/D and Z/H combinations.

A more rapid decrease in g_s was induced under waterlogging in all grafting combinations and slightly delayed in the Z/B combination. This suggests that the B rootstock may be more tolerant to waterlogging stress than the D and H rootstocks and that the rootstock may influence the response of the grafted cultivar when exposed to waterlogging stress.

Rootstocks with different waterlogging tolerance were found to influence the photosynthetic capacity of the scion, maintaining it for a longer time under waterlogging stress in peach (Zhang et al., 2023), and to improve the physiological, biochemical, and molecular responses of the scion in kiwifruit (Bai et al., 2022). This influence, which is only reflected in the Z/B combination, may be of great importance in increasing the potential tolerance of kiwifruit to excess water in poorly structured soils and should therefore be taken into account in the genotypic selection of the rootstock. However, the waterlogging tolerance of the B rootstock is not fully reflected in the grafting combination.

In particular, g_s followed by a rapid decrease in A was previously observed in 3-month-old plants of ‘Hayward’ under waterlogging stress, suggesting a high sensitivity of this rootstock to waterlogging conditions (Li Z. et al., 2021). Although no information is available specifically for the D rootstock, the data analyzed suggest that the D and H rootstocks behave similarly under waterlogging stress, identifying them as waterlogging-sensitive rootstocks. Both rootstocks belong to *A. chinensis* var. *deliciosa* which has been found to have a low tolerance to waterlogging (Youn-Seop et al., 2008; Bai et al., 2019; Kataoka et al., 2021; Beppu et al., 2022). The observed physiological behavior of the B rootstock, which was not affected by the 9-day waterlogging treatment, was consistent with previous studies showing that A, g_s , and E in *A. macrosperma* were maintained at relatively high levels even under waterlogging stress (Kataoka et al., 2021; Beppu

et al., 2022), together with high root activity (Bai et al., 2019). Furthermore, Kataoka et al. (2021) found that grafting ‘Hayward’ onto *A. macrosperma* rootstock allowed greater tolerance to be expressed in the grafted kiwifruit scion. This is consistent with the results of the present study, where the physiological responses of the scion grafted on the B rootstock to waterlogging stress were delayed in time compared to other grafting combinations.

Maintaining an efficient and effective photosynthetic activity and undamaged photosynthetic apparatus is extremely important for plant growth and development (Zhang et al., 2023). Photosynthetic gas exchange parameters can be used to assess plant tolerance to waterlogging. Waterlogging stress can induce stomatal closure, which limits leaf gas exchange and leads to reduced CO₂ absorption, thereby affecting photosynthetic rate (Pan et al., 2021). The initial reduction in A is reported to be highly correlated with stomatal closure, but after prolonged periods of waterlogging, the rate of A also decreases due to the inhibitory effects on the photosynthetic process (Kozłowski, 1997).

By analyzing the different trends in C_i values, it is possible to detect the presence of mechanisms other than g_s (non-stomatal factors) that influence the assimilation process and thus photosynthetic rates. Lower C_i values in the WL vines of the Z/D and Z/H combinations supported that the leaves were CO₂-limited due to lower g_s. Conversely, Pimentel et al. (2014) found increased C_i values in waterlogged plants of *Prunus* species, suggesting that the leaves were not CO₂-limited and that lower A was determined by non-stomatal factors. Similar results were instead found by Baldi et al. (2024) in ‘Hayward’ kiwifruit, where high reductions in A were observed in plants exposed to waterlogging, mainly associated with significantly lower g_s, resulting in lower C_i values. The higher C_i value found in WL vines of the Z/D combination at DAW 4 indicated the occurrence of non-stomatal factors, identifying a later stage of waterlogging stress when photosynthetic metabolism was almost irreversibly damaged, as indicated by very low A. Instead, WL vines of the Z/H combination maintained a lower C_i also at DAW 4, confirming the effect of lower g_s on the assimilation process and thus no damage to the photosynthetic apparatus already at this time. On this basis, a slightly lower sensitivity of the Z/H combination to waterlogging stress compared to the Z/D combination is suggested.

The results concerning WUE_i are consistent with Xu et al. (2022), who showed that some peach rootstocks maintained high levels of WUE before gradually declining until the end of the stress and that very low values may indicate irreversible damage to photosynthetic properties. Lower WUE_i values in WL vines of the Z/D combination at DAW 4 may indicate the beginning of a damage process to the photosynthetic system.

Mature leaves of kiwifruit vines are characterized by large dimensions and an orbicular shape in the D and H rootstocks and in the grafted yellow-fleshed cultivar, features that contribute to increased leaf temperatures (Smith et al., 1989). Significant differences in T_{leaf} between WW and WL vines can be attributed to significant reductions in g_s and E , which determine the attainment of lethal temperatures in parts of the leaves that can be irreversibly damaged (Smith et al., 1989). In the present study, the physiological responses to waterlogging stress varied between the three kiwifruit rootstocks and grafting combinations. Early reductions in leaf gas exchange under waterlogging stress were observed in the D and H rootstocks compared to the B rootstock, which was not affected throughout the experiment. In particular, the reductions were more pronounced in the grafting combinations where the influence of the rootstock on the scion was evident.

5.4.2 Image-based parameters to phenotype for waterlogging stress

Many studies have used image analysis to investigate the responses of plants to various abiotic stresses (e.g., drought, heat, and salt stress) (Briglia et al., 2019; 2020; Kim et al., 2020; Carvalho et al., 2021). Non-destructive and non-invasive phenotyping assessment of waterlogging responses using a high-throughput plant phenotyping platform in fruit tree crops has been poorly addressed, and the results obtained in the present study represent a novelty for the kiwifruit crop.

The detrimental effect of waterlogging stress on plant growth and development is well known (Manghwar et al., 2024), and plant biomass is an important trait assessed to investigate the waterlogging response of plants (Langan et al., 2022). Among the morphological parameters that can be assessed non-destructively by image analysis, PSA and S may be relevant indicators to evaluate kiwifruit vine development and changes in leaf density and spread as influenced by waterlogging. Traditional phenotypic observations can be influenced by cultivar-specific traits such as leaf surface area and maturity (Zhang et al., 2023), which in turn affect their response to waterlogging stress. The waterlogging tolerance or sensitivity of rootstocks can directly influence scion growth and development in kiwifruit (Bai et al., 2019).

Significant differences in PSA and S between rootstocks can be attributed to differences in leaf number, size, shape, and anatomy, particularly distinguishing the B rootstock from the D and H rootstocks. The reductions in PSA that occurred in the D and H rootstocks under waterlogging showed an effect of waterlogging stress on total vine area. Previous phenotyping studies have used PSA or similar indices of plant biomass in several crops and found similar deleterious effects of drought stress on plant aerial mass (Danzi et al., 2019; Briglia et al., 2020; Kim et al., 2020; Genangeli et al., 2023; Petrozza et al., 2023), reinforcing the issue that waterlogging stress affects leaves in a similar way to drought stress (Topali et al., 2024). The PSA evaluated in the grafting

combinations confirmed the results found in the rootstocks, with a more pronounced effect of waterlogging stress on the aerial mass of the vines of the Z/D and Z/H combinations.

In the present study, the most sensitive D rootstock showed a higher S under waterlogging stress, as determined by the lower convex hull area values of WL vines compared to WW vines, although waterlogging induced a decrease in PSA. Higher values of S were previously observed in a drought experiment carried out on tomato plants, where drought-stressed plants lost turgor and reduced their convex hull area, resulting in increased S (Janni et al., 2019). However, reduced S has also been suggested as a consequence of water stress and could be determined by decreasing the plant area pixels while keeping the convex hull area constant (Petrozza et al., 2023). In the case of the grafting combinations, S values were lower in the WL vines compared to the WW vines, with a decreasing trend with prolonged waterlogging exposure in the Z/D and Z/H combinations, indicating a gradual wilting of the leaves, which is consistent with considering lower S values as a consequence of stress. Petrozza et al. (2023) used S as an index of the differential response of tomato plants to drought stress and reported lower S in sensitive plants under drought conditions, which was explained as a consequence of the reduced plant pixel area due to greater wilting of drought-sensitive plants. S is then a relative index that varies between species and even cultivars according to the specific behavior of plants to a given stress.

Waterlogging stress often causes leaf chlorosis, wilting, and yellowing (Langan et al., 2022), reducing green leaf area (Ren et al., 2023). The leaves turn yellow and develop necrosis as a result of prolonged exposure to waterlogging at the roots, leading to abscission (Topali et al., 2024). Furthermore, changes in the balance of leaf catabolism and anabolism processes during waterlogging stress have been reported to cause a decrease in leaf chlorophyll content (Zhang et al., 2023), which could contribute to reduced photosynthesis and leaf color change, which gradually turn from green to yellow with prolonged exposure to waterlogging. The RGB images have great potential not only for morphological studies, including parameters such as PSA and S, but also for leaf color estimation. In particular, leaf chlorosis, which is an indication of the chlorophyll degradation and contributes to a decrease in A, can be detected by measuring the number of pixels in the green and yellow regions of the hue channels. Significant differences in HUE in WL vines of the Z/D and Z/H combinations may indicate the beginning of a process of leaf chlorosis induced by waterlogging. Assessment of leaf color, degree of yellowing, or loss of greenness has been reported as a promising tool to detect the occurrence of drought stress, as an increasing fraction of the yellow color class was associated with increasing drought in a grapevine experiment (Briglia et al., 2019). The SI was used to indicate the senescence status of the vines.

No significant differences in SI were found between the irrigation treatments, although an increasing trend was observed in WL vines of all rootstocks and Z/D and Z/H combinations.

While the effects of waterlogging were registered almost immediately in the RGB morphometric parameters, the color changes appeared with a delay and with greater variability between the vines, in agreement with other studies (Janni et al., 2019; Genangeli et al., 2023). The results indicate that the RGB image-based parameters that most accurately represented the differential response of kiwifruit rootstocks and grafting combinations to waterlogging were plant PSA and HUE color class. These parameters can be effective indicators for screening for more tolerant kiwifruit genotypes using non-destructive and non-invasive plant phenotyping.

The water content of plant tissues is known to influence spectral reflectance, and therefore, non-destructive techniques such as those based on NIR wavelengths are useful for assessing plant water status (Kim et al., 2020; Danzi et al., 2022). NIR intensity values showed an increasing trend (lower water content) in all WL vines of the grafting combinations, suggesting that waterlogging affected the water status of the vines as detected by NIR images. The water content of plant tissues was then reduced under waterlogging stress, which has been reported as an effect of waterlogging on plants (Sathi et al., 2022). The occurrence of partial leaf wilting and increased leaf surface necrosis area contributed to the reduction in water content of tissues that began to dry out.

Physiological measurements and RGB analysis identified the Z/D combination as the most sensitive to waterlogging stress, followed by the Z/H combination. Furthermore, 4 days of waterlogging stress can be identified as the time when a more severe stress occurred for the Z/D and Z/H combinations, as all image-based parameters began to change from the control values. Although the Z/B combination showed a delayed decrease in physiological activity compared to the other combinations, it did not show any changes in morphometric and colorimetric parameters under waterlogging stress. RGB image-based results confirmed that the B rootstock enhanced the tolerance of the scion to waterlogging stress.

5.5 Conclusions

Waterlogging and root hypoxia conditions are predicted to become more severe due to anomalous climatic conditions, and represent a serious threat that is increasingly affecting kiwifruit cultivation. Novel results are presented on the assessment of the waterlogging response of different kiwifruit rootstocks and grafting combinations by combining traditional physiological measurements with high throughput phenotypic analysis.

Physiological performances and image-based parameters were negatively affected by soil waterlogging, but to different extents in the kiwifruit rootstocks and grafting combinations studied.

D and H rootstocks showed an early reduction in stomatal conductance and photosynthetic rates, and an increase in leaf temperature already during the first days of waterlogging stress, confirming that these parameters are effective indicators of altered behavior caused by waterlogging, as by other abiotic stresses. RGB and NIR image analysis, through the evaluation of parameters such as projected shoot area, leaf color, and plant tissue water content, supported the waterlogging sensitivity of the D and H rootstocks and their combinations and provided promising indicators to be used in the evaluation of the waterlogging response of kiwifruit vines. Instead, B rootstock was unaffected by 9 days of waterlogging stress in both physiological and phenotyping parameters. Waterlogging effects were more pronounced in the yellow-fleshed kiwifruit cultivar grafted on D and H rootstocks than in the cultivar grafted on B rootstock, suggesting an influence of the rootstock on the scion. However, stomatal conductance also decreased in the Z/B combination, suggesting that the B rootstock is more tolerant to waterlogging, but not able to allow the grafted cultivar to cope with prolonged and adverse soil conditions.

More efforts should be made to adopt proper orchard management and to promote breeding research for the selection of well-adapted plant material (rootstocks and cultivars). The present study reveals the suitability of RGB morphometric and colorimetric parameters analyzed on kiwifruit vines under waterlogging stress as promising indicators. In conclusion, physiological and phenotyping assessment can be an effective methodology for screening for more waterlogging tolerant genotypes for the kiwifruit crop.

5.6 References

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6. General conclusions

The present work contributes to a better understanding of the eco-physiological responses of yellow-fleshed kiwifruit grown in a Mediterranean environment to water stress, and to the implementation of advanced methodologies for irrigation management and genotype screening.

On the one hand, combining plant measurements (e.g., stem water potential and leaf gas exchanges) with continuous soil water content monitoring enables the identification of soil moisture thresholds, providing suitable soil water conditions for optimal vine physiological performance. In this study, the readily available water that can be used before the plant water deficit occurs is approximately 11% of the available water in the soil volume wetted by localized irrigation. The dynamics of root water uptake highlight the different contribution of each soil layer to meeting the transpiration needs of the vines. The superficial soil layer (from 0 to about 30 cm) is the most interested in root water uptake and should therefore be carefully monitored to keep the available water within the recommended levels (approximately 20% of the available water can be used in this soil layer).

In addition, the integration of continuous plant measurements (e.g., sap flow and trunk water potential) with mechanistic plant modelling allows the daily derivation of plant hydraulic parameters that are otherwise challenging to measure. The estimated hydraulic capacitance and hydraulic resistance of kiwifruit vines correlate with trunk water potential, a reliable indicator of plant water status, and reveal drought stress responses in kiwifruit. The developed modelling approach can be tested and transferred to various fruit crops and has the potential to implement effective practical tools for irrigation management and scheduling in fruit crops.

On the other hand, the combined use of phenotyping analysis with physiological measurements allows a comprehensive evaluation of the behavior of different rootstocks and scion/rootstock combinations and screening for more tolerant genotypes to abiotic stress (e.g., water stress) in kiwifruit. Of these, the 'Bounty' rootstock is the least susceptible to water stress compared to the other rootstocks studied (i.e. 'Hayward' and 'D1') and this is reflected in the scion. This is of great importance in the context of the growing threat of 'KVDS', where the search for tolerant rootstocks has received increasing attention in recent years. However, more tolerant genotypes, such as 'Bounty', recently introduced as a rootstock in Italian plantations, are not able to cope with long-term adverse soil and environmental conditions, so optimizing kiwifruit orchard management remains essential to ensure kiwifruit resilience and productivity.

In conclusion, precision irrigation is key to the success of kiwifruit production and more efforts should be made to adopt appropriate orchard management practices to counter the effects of climate change and improve orchard resilience. However, breeding programs for more tolerant genotypes are becoming increasingly important to improve stress tolerance and expand kiwifruit production to other areas of a country with less suitable conditions. The findings of the present work certainly open the way for future research aimed at developing sustainable and precise irrigation strategies and selecting new, more resilient genetic material in kiwifruit to face the current challenges posed by climate change.